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SOIL SCIENCE

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THE NATURE OF SOIL ACIDITY AS AFFECTED BY THE SiO_2-SESQUIOXIDE RATIO[1]

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The modern trend of thought regarding the nature of base exchange reactions has rather definitely related soil acidity to the absorptive properties of the soil. There have been many different views presented, however, to explain the exact relation between these exchange properties and soil acidity. Although the investigations of Bradfield, Kelley, Mattson, and Truog in this country and the work of the Europeans, Gedroiz, Hissink, Odén, Page, and others, have solved a number of problems related to soil acidity, many conflicting ideas that need to be clarified have arisen from their studies.

It is the purpose of this paper to show the differences in the acids from various soils and to discuss the relationships existing between these acids and the kind and extent of weathering under which the soil has developed; to show the relation of the nature of the soil acids to the chemical composition of the soil colloidal material as expressed by the SiO_2-sesquioxide ratio; and to discuss the reliability of the methods for determining the chemical composition and nature of the soil exchange complex.

REVIEW OF LITERATURE

Most soil investigators agree that base exchange and soil acidity phenomena originate in the complex ferro- and alumino-silicates found in the soil colloidal material. Bradfield (4) in his original work on the chemical reactions of a colloidal clay, advanced the theory that the colloidal clay of an acid soil is a true acid which ionizes to give a definite H-ion concentration. He considered these colloidal particles as large complex ions. The titration curve of the soil acid showed the characteristic properties of a monobasic acid. It was well buffered throughout the entire pH range. Although it acted similarly to a monobasic acid, Bradfield considers its ionization as representing a series of mass action relations as expressed by the equation:

$$\frac{[H^+]\,[X^-]}{[HX]_n} = K_a, K_b, K_c \text{ ——— } K_n$$

where K_a, K_b . . . . K_n represent the dissociation constants of a series of acids. The explanation for obtaining the typical monobasic acid titration curve in lieu of this series of acids is based upon the fact that a mixture of acids with unlike dissociation constants may give the

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[2] Associate and assistant soil chemist, respectively.

typical monobasic curve if their respective constants are close enough together so that their individual curves overlap. The relative amount of any one acid would probably determine the exact shape of the curve. One of the writers (3) has shown that the nature of the titration curve or any particular soil acid is dependent upon the valance and hydration of the cation of the base with which the acid is titrated. Further work by Bradfield (5) has confirmed his original theory that soil acidity is due to complex colloidal alumino-silicic acids that dissociate in a similar manner to ordinary monobasic acids. Recent work by one of the writers (3) has shown that the physico-chemical properties of the soil colloidal material are functions of the degree of dissociation of these complex colloidal acids and their salts.

Kelley and Brown (11), from an analysis of their work on base exchange, advance a similar explanation for soil acidity, stating that the H-ion concentration of the soil is determined by the products of dissociation of the exchange complex, the hydrolytic products of the complex, and the buffer properties of the soil. They consider the replaceable bases in a soil as being the cations of salts of one or more alumino-silicic acids. Page (17) states that all soil acidity is essentialy of one kind, namely, that it is due to exchangeable hydrogen. The absorbing complex of the soil is considered as being an "acidoid," an insoluble colloidal acid, which is associated with surface active hydrogen and basic cations. For any given soil this complex possesses a fairly well-defined saturation capacity. Odén (16) accepts the views of Page and Bradfield, stating that these "acidoid" elements are a result of the weathering processes and that each soil will have a relatively constant pH due to the relatively constant rainfall and the composition of the mineral part of the soil. The fixation of H ions is attributed to the SiO_2 in the exchange complex, and the fixation of OH ions to the Al_2O_3 and Fe_2O_3.

Kerr (12, 13) has shown that the exchange complex is composed of definite chemical compounds having a characteristic crystalline form. He comes to the same conclusion as Bradfield and others that base exchange and soil acidity are due to complex alumino-silicates. He further concludes, however, that the predominant, if not the only, mineral in the inorganic exchange complex is a monobasic alumino-silicic acid corresponding to the formula

$$H_2O\ (Al_2O_3 \cdot 6\ SiO_2)\ 8H_2O$$

Irrespective of the nature of weathering processes, this compound is considered as being the sole acid that imparts acidity to a soil. The difference between the acidity of different soils is one of degree and not of kind. Joffe and McLean (10) suggest that various soils possess the same type of colloidal substance that varies only in its degree of dispersion. The results in this paper will aid in clarifying these divergent opinions.

The chemical composition of the soil colloidal material as expressed by the SiO_2-sesquioxide ratio varies considerably in different soils. Robinson and Holmes (20) have reported the results of numerous analyses of soil colloids which indicate that the extent of leaching of various soils is responsible for these variations in composition. Since the soil colloidal material is chiefly composed of the products of weathering of various soil-forming minerals, its composition varies considerably depending upon the kind and extent of weathering. Colloidal material extracted from soils developed under humid conditions and fairly high temperatures tend to have a low SiO_2-sesquioxide ratio because the silica is removed from the soil by the weathering processes. On the other hand, the colloidal material from soils weathered in a humid climate with moderately low temperatures tends to have a high ratio, because aluminum and iron are leached away. Therefore, the soils from the north (podzolic soils) have a higher ratio than those weathered under tropical or subtropical conditions (lateritic soils). Gile (9) states that different soil colloidal materials are of the same general nature, varying only in their SiO_2-sesquioxide ratio. The nature of the colloid is changed by varying the content and nature of the silica.

Numerous investigators have shown that the properties of the soil colloidal material vary with the SiO_2-sesquioxide ratio. Anderson and Mattson (2) have shown that the heat of wetting, and the absorptive capacity of the soil for NH_3 and malachite green are proportional

TABLE 1

Description of soils used in this study

SOIL NUMBER	SOIL TYPE	LOCATION	DEPTH	REMARKS
			inches	
386	Decatur silt loam	Alabama	0–6	Highly weathered soil from an old limestone valley
557	Lintonia silt loam	Arkansas	0–6	River terrace soil, moderately weathered
671	Cory silt loam	Illinois	0–6	Highly weathered soil from the Illinoian drift
674	Cecil clay loam	Alabama	0–6	Highly weathered soil from silicate rocks in the Piedmont Plateau
675	Norfolk sandy loam	Alabama	0–6	Soil weathered from unconsolidated sandstone in the Coastal Plain, not weathered as highly as soils 386 and 674
676	Colby silt loam	Wisconsin	0–6	Loessial soil associated with late Wisconsin Drift, moderately weathered
679	Miami silt loam	Wisconsin	0–6	Moderately weathered limestone soil from late Wisconsin Drift
680	Greenville fine sandy loam	Alabama	0–6	Soil from the Coastal Plain of limestone origin, weathered in a similar manner to soil 675
707A*	Susquehanna clay	Alabama	0–8	Grayish-brown, loamy fine sand
707B	Susquehanna clay	Alabama	8–20	Bright red, well-oxidized clay
707C	Susquehanna clay	Alabama	20–30	Sticky, heavy, blue-mottled, red clay
707D	Susquehanna clay	Alabama	At 10 feet	Blue clay, slightly mottled with yellow
710A†	Oktibbeha clay	Alabama	0–2	Brown clay loam
710B	Oktibbeha clay	Alabama	2–6	Yellow-brown clay
710C	Oktibbeha clay	Alabama	6–12	Reddish-brown, mottled with yellow, heavy clay
710D	Oktibbeha clay	Alabama	12–18	Yellow, mottled with red, heavy clay
722A†	Eutaw clay	Alabama	0–4	Light brown clay
722B	Eutaw clay	Alabama	4–14	Yellow, mottled olive-green and gray, sticky clay
742A†	Lufkin clay	Alabama	0–6	Gray-brown, mottled clay
742B	Lufkin clay	Alabama	6–15	Sticky, gray clay
743	Putnam clay	Missouri	14–24	Layer of accumulation of the Putnam silt loam, well-weathered prairie soil

* The Susquehanna clay occupies the hills and rolling areas on the inner border of the Coastal Plain, originating from clay deposits of marine origin. It is moderately weathered in the surface horizons.

† The Lufkin, Eutaw, and Oktibbeha clays represent soils from the coastal plain Black Belt. They are of marine origin and have been deposited upon a bed of limestone known as the Selma chalk. Their extremely impervious nature has hindered the weathering processes. The Lufkin is the most highly unweathered of these soils. The Oktibbeha is weathered the most. The Eutaw occupies an intermediary position in regards to weathering.

to this ratio. Parker and Pate (18) report that there is a good correlation between the SiO_2-sesquioxide ratio of the soil colloids and their exchangeable base content. Colloids with a high ratio tend to have more exchangeable bases. Mattson (15) has fairly definitely established that the capacity of the exchange complex to absorb cations increases with this ratio. The absorptive capacity of the soil for cations increases after the removal of the free sesquioxides. Adsorption of anions increases with a decrease in the ratio. He attributes the absorption of anions to the free sesquioxides that the soil may contain. Anderson also states that the absorptive capacity and exchangeable base content of soils are dependent upon this ratio. Although these data do not show an exact relationship between the base exchange properties of the soil colloids and the SiO_2-sesquioxide ratio, undoubtedly the absorptive properties of soil colloids are dependent in some way upon this ratio.

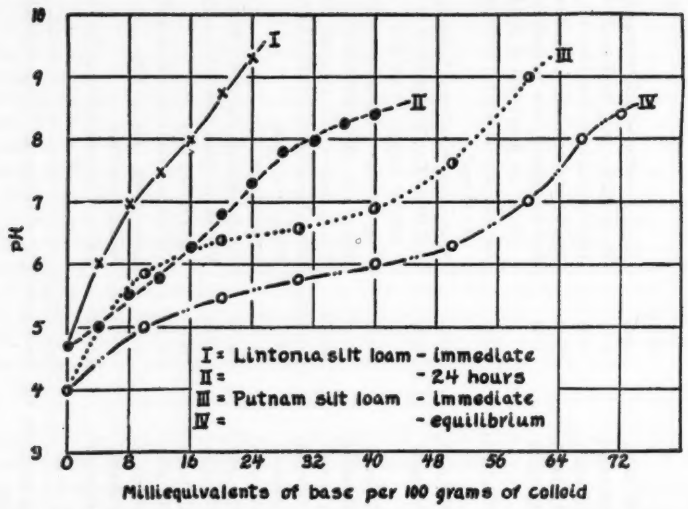

FIG. 1. THE EFFECT OF TIME ON THE TITRATION CURVES OF COLLOIDAL CLAY ACIDS

EXPERIMENTAL

Colloidal material extracted from 27 different soils, by the method of Bradfield (4), was used in this investigation. Since several of these soils were of the same soil series and type, the results on only 21 soils are reported in this paper. The curves for soils from the same series were practically identical in nature In order to show more clearly the wide differences in these soils with respect to origin and the kind and extent of weathering, a brief description is given in table 1.

Two grams of colloidal material extracted from each of these soils were electrodialyzed in a Bradfield three-compartment cell until all of the exchangeable cations were removed. The contents of the cathode chamber were analyzed for exchangeable cations. The electrodialyzed colloids were transferred from the cell to Erlenmeyer flasks. A 1 per cent colloidal suspension was obtained after the cell was rinsed out. Then suspensions were titrated electrometrically

with 0.1 *N* NaOH, the hydrogen electrode being used. The suspensions were shaken on a mechanical shaker for 2 hours after the addition of the base and allowed to stand for approximately 18 hours in order to attain equilibrium. Readings taken immediately after the addition of NaOH were found to be unreliable because equilibrium between the base and the acid clay had not been obtained. This is clearly shown in figure 1. Bradfield (6) has shown that this method of removing all of the exchangeable bases from soil colloids and titrating the resulting H-colloid with a base not only gives the total exchange capacity

TABLE 2

Soil acidity data on the soils used in this investigation

SOIL NUMBER	SOIL TYPE	SiO_2 / R_2O_3	TOTAL EXCHANGE CAPACITY M.E. PER 100 GM. COLLOID	pK OF SOIL ACID	DEGREE OF DISSOCIATION OF SOIL ACID*	EXCHANGE CAPACITY ÷ pK VALUE
					per cent	
386	Decatur silt loam	1.73	11	6.15	23.0	1.80
557	Lintonia silt loam	2.60	22	5.75	4.5	3.82
671	Cory silt loam	3.20	22	5.65	40.4	3.90
674	Cecil clay loam	1.60	13	6.45	6.1	2.00
675	Norfolk sandy loam	1.92	22	6.85	8.1	3.20
676	Colby silt loam	3.18	32	6.10	8.8	5.25
679	Miami silt loam	3.97	30	6.25	11.8	4.80
680	Greenville fine sandy loam	1.62	20	6.50	8.8	3.10
707A	Susquehanna clay	2.27	28	5.85	25.2	4.80
707B	Susquehanna clay	2.11	36	6.35	14.0	5.70
707C	Susquehanna clay	3.25	55	6.25	8.1	8.80
707D	Susquehanna clay	3.50	46	5.20	7.8	8.80
710A	Oktibbeha clay	1.81	34	5.20	41.4	6.50
710B	Oktibbeha clay	1.83	34	5.75	46.4	5.90
710C	Oktibbeha clay	1.90	22	6.05	7.2	3.65
710D	Oktibbeha clay	1.92	22	6.30	6.4	3.50
722A	Eutaw clay	2.18	27	5.55	11.7	4.70
722B	Eutaw clay	2.31	33	5.15	15.1	6.40
742A	Lufkin clay	3.85	30	6.00	11.8	5.00
742B	Lufkin clay	3.81	30	5.70	16.6	5.30
743	Putnam clay	3.20	57	5.75	22.0	9.90

* Determined at the pH of the pure acid colloidal clay.

of the colloid, but also determined the dissociation constant of the soil acid.

The SiO_2-sesquioxide ratio was determined in the usual way. TiO_2 was not included with the Al_2O_3.

RESULTS

The titration curves of these soil acids are graphically shown in figures 2, 3, 4, 5, and 6. The SiO_2-sesquioxide ratio, exchange capacity, pK values and the degree of dissociation of the acids are given in table 2. These curves show

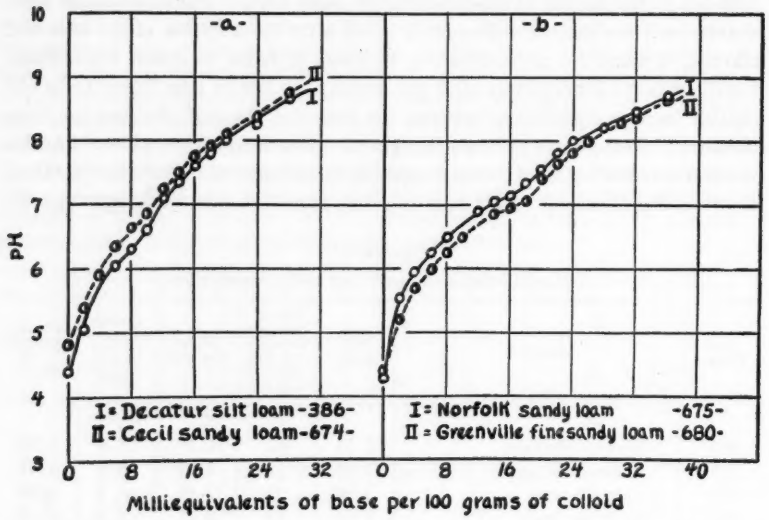
-a-
-b-
pH
I = Decatur silt loam -386-
II = Cecil sandy loam -674-
I = Norfolk sandy loam -675-
II = Greenville fine sandy loam -680-
Milliequivalents of base per 100 grams of colloid

FIG. 2. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM VARIOUS SOUTHERN SOILS

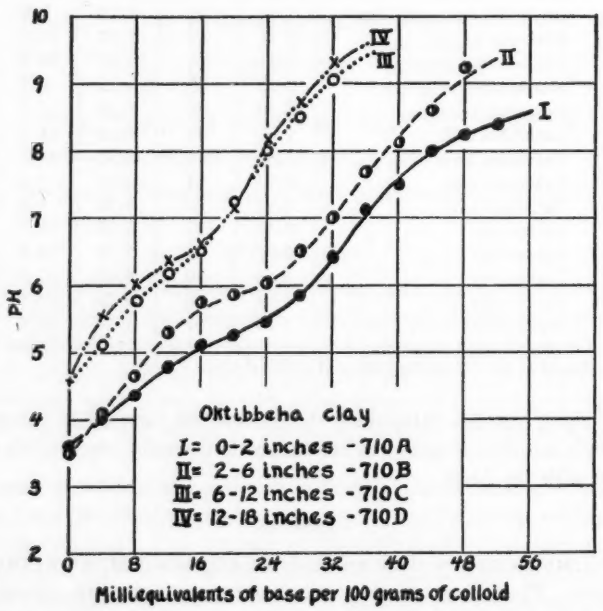
pH
Oktibbeha clay
I = 0-2 inches -710A
II = 2-6 inches -710B
III = 6-12 inches -710C
IV = 12-18 inches -710D
Milliequivalents of base per 100 grams of colloid

FIG. 3. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM OKTIBBEHA CLAY

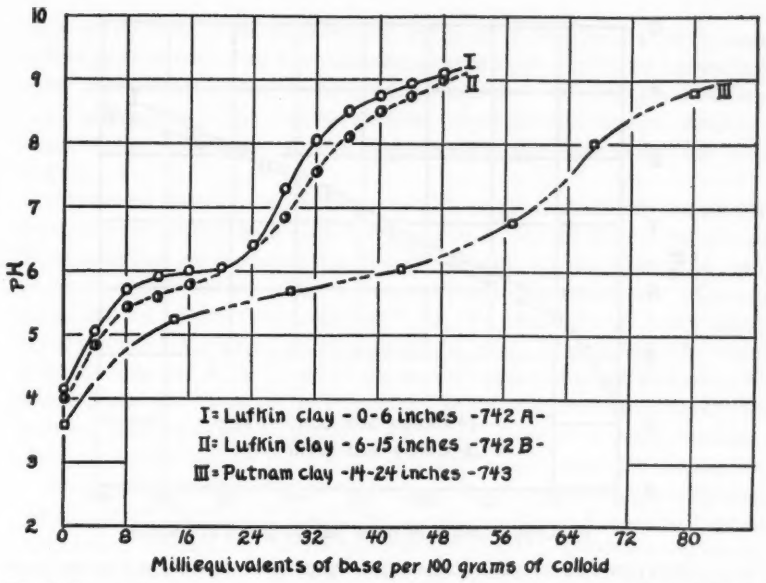

FIG. 4. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM DIFFERENT CLAYS

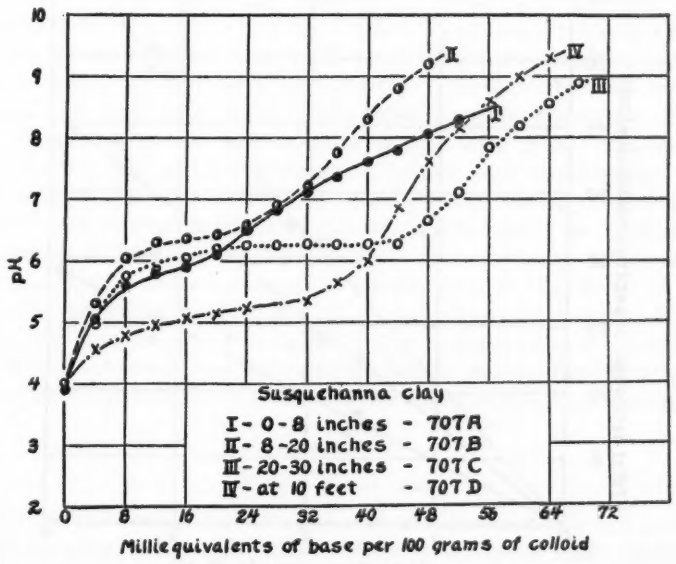

FIG. 5. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM DIFFERENT HORIZONS OF SUSQUEHANNA CLAY

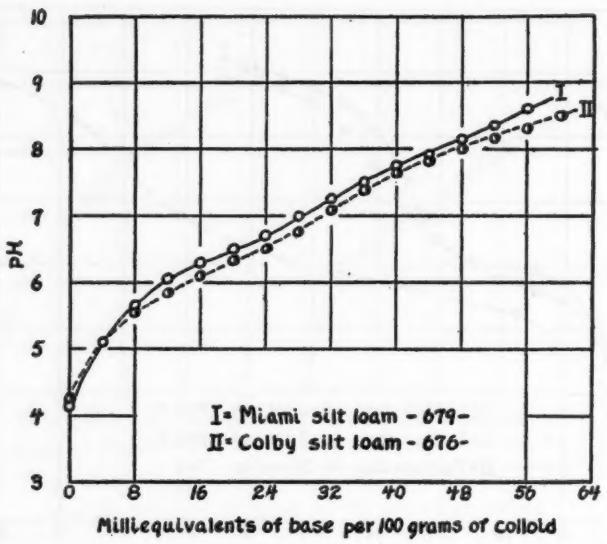

FIG. 6. TITRATION CURVES OF COLLOIDAL CLAY ACIDS EXTRACTED FROM VARIOUS SILT LOAMS

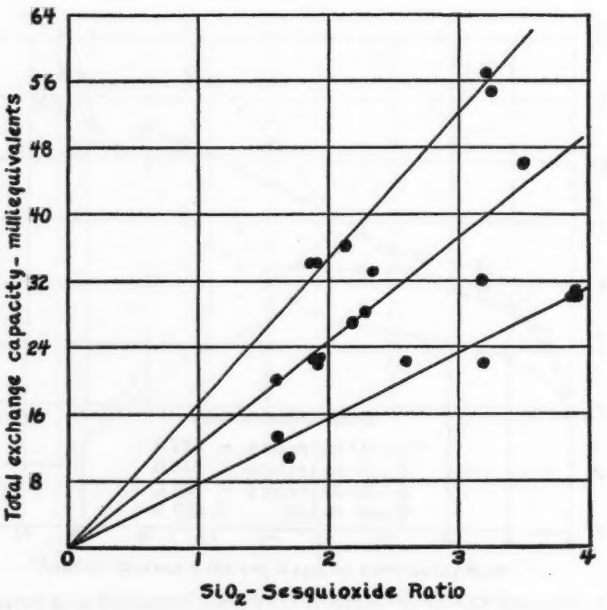

FIG. 7. THE RELATION OF THE TOTAL EXCHANGE CAPACITY TO THE SiO_2-SESQUIOXIDE RATIO

very clearly that the nature of the soil acid in all of these soils is not the same. Although all of the curves have the same general shape, similar to a monobasic acid, their respective slopes and the pH range over which they are buffered vary considerably. However, the titration curves of soils that have weathered under similar conditions and to approximately the same extent are quite similar.

If these curves are compared with the SiO_2-sesquioxide ratio of the soil colloids, several general tendencies are manifested. In the first place, if only those soils that are moderately or highly weathered are considered, the total exchange capacity of the colloid increases with the SiO_2-sesquioxide. This has been observed by other investigators (12 18, 19). Secondly, the buffer capacity of the colloidal material increases with this ratio. This is especially manifested by soils 707 A, B, C, and D and soil 743 when compared with soils 386, 674, 675, and 680. Finally, the strength of the soil acid, that is, its dissociation constant, also tends to increase with this ratio. These three general relationships can be drawn from the existing data with a fair degree of accuracy.

The relative strengths of these soil acids can best be expressed by multiplying the amount of acid present, that is, the exchange capacity of the colloid, by its dissociation constant. Since the dissociation constants of these acids are expressed in this study by their pK values, the exchange capacity is divided by this value. If the resulting factor is plotted against the SiO_2-sesquioxide ratio, there appears to be no one direct relationship between them. However, there appears to be three different groups of soils in which there tends to be a fairly good relationship between these two factors. If the total exchange capacity is plotted against the SiO_2-sesquioxide ratios (fig. 7) similar relationships are obtained. The same three groups of soils appear to remain intact even though the pK values of the soil acids do not fall in these same classes. The work of Mattson (14), who used five different soils, indicates that there is a direct relation between the SiO_2-sesquioxide ratio and exchange capacity. Anderson's data (1) with seven soils show that this relationship tends to be logarithmic. The results of Parker and Pate (18) with colloids from 14 soils may be interpreted in such a way as to show that this relationship is either logarithmic, or that there are two groups of colloids in which a direct relationship is obtained. All of the existing data on the relation of the absorptive properties of soil colloids to the SiO_2-sesquioxide ratio indicate that there is some relationship between these two factors, even though its exact nature cannot be accurately established.

DISCUSSION

Nature of soil acids

It is apparent from an analysis of these titration curves and the data in table 2 that the nature of soil acids varies considerably in different soils. Highly or moderately weathered soils with a high SiO_2-sesquioxide ratio tend to have a higher buffer capacity and a smaller pK value than those that have not wea-

thered so thoroughly. However, there are several outstanding exceptions to this more or less general tendency. For example, if soils 676, and 679 (fig. 6), soils 707C and 707D (fig. 5), and soil 743 (fig. 4) are compared, it is apparent that even though they have a SiO_2-sesquioxide ratio > 3 and represent soils that are fairly well weathered, there are significant differences beween their titration curves. Their buffer capacities and the strength of their acids are different. If the conditions of weathering under which these particular colloids have developed are considered, some light is thrown upon the cause of these differences. Soils 676, and 679 are the surface horizons from soils that have developed under the podzolic type of weathering. In this type of weathering, aluminum and iron leach away from the upper layers of the soil profile and tend to precipitate or settle out in the lower horizons where they encounter a less acid medium in their percolation downward. This tends toward the formation of a complex in the surface soil that will be high in silica, either as a part of the exchange complex or as free SiO_2. On the other hand, soil 743 represents the layer of accumulation from a podzolic type of soil. This horizon contains the highest percentage of colloidal material. Materials in suspension that percolate downward from the surface horizons accumulate in this zone. Therefore, its exchange complex should be expected to be different from that of the surface horizons from which materials have been removed.

Numbers 707C and 707D represent soils that have weathered under the lateritic type of weathering in which silica tends to be carried away and aluminum and iron tend to remain behind. These two soils are the least weathered horizons of this Susquehanna profile. However, several interesting relationships are manifested. Soil 707D has a SiO_2-sesquioxide ratio of 3.50. The ratio of 707C is 3.25 while that of the horizon above it is only 2.11. This shows very clearly that as the soils of the south become more highly weathered, silica is removed from the complex. There appears to be a significant change in the type of acid as the weathering processes alter these soils. From an analysis of the data of these six soils, all of which have somewhat similar SiO_2-sesquioxide ratios, but represent different types and degrees of weathering, it is apparent that there are different types of soil acids in these particular soils. This fact is further substantiated if the highly weathered soils of the south, soils 386, 674, 675, 680, 707A, and 707B, are compared with these soils previously mentioned or with the soils of the coastal plain Black Belt, soils 710, 722, and 742. These differences cannot be explained by variations in the SiO_2-sesquioxide ratio, as will be brought out later in the discussion. Pierre and Scarseth (19), in studying the relation of percentage base saturation of soils to their pH values, state that the differences in soil acids cannot be attributed to variations in this ratio. They conclude that the percentage base saturation of soils at like pH values is related to the degree of dissociation of the soil acids. The degree of dissociation of any soil acid, of course, is a function of its dissociation constant and the quantity of acid present, as previously mentioned. Its relative value can be calculated at any pH value by dividing the

measured hydrogen-ion concentration by the total amount of hydrogen present (exchangeable hydrogen).

The exchange complex of any soil and, therefore, the type of soil acid, may originate in any one or all of three ways. First, weathering processes may remove certain constituents from the original alumino-silicate minerals which are leached away leaving behind a complex that would be characteristic of the kind of weathering and independent of the parent material. Secondly, the colloidal oxides of Al, Fe, and Si that arise from a partial decomposition of the original complex may mutually flocculate and form an entirely different type of complex. Finally, there may be a precipitation of Al, Fe, and Si from solution that would give another type of complex. These three possibilities would allow the formation of different complexes dependent upon the kind and extent of weathering. This conception is also held by Gedroiz (8). Mattson (14), working with artificial zeolites, found that the nature of his precipitates depended upon the H-ion concentration. Precipitates having a high SiO_2/Al_2O_3 were obtained in the presence of Ca and Mg ions. In the absence of bases, but in a neutral or slightly acid medium the precipitate consisted largely of sesquioxides. Burgess and McGeorge (7) were able to obtain different zeolites dependent upon the reaction at which they were formed. Ross (21), in studying the mineralogy of clays, states that clay minerals may form by weathering certain elements from an old mineral; by complete replacement of a mineral; by crystallization of colloidal material; by direct precipitation from solutions; and by a reaction between older minerals and the soil solution. He further states that there is a relation between the type of clay mineral and the climatic or other physical conditions under which it is formed and that it would have different chemical and physical properties. He found, however, that there appeared to be a "dominant clay-forming mineral" of the beidellite type.

The data here presented do not confirm the conclusions of Kerr (13), who states that there is only one soil acid. However, it may be that the soils from which he extracted the "true" exchange complex were of such a nature that wide differences were not manifested. Soils from the South may compare with those from the North if the extent of weathering has not been considered.

The nature of the soil acid appears to be so closely associated with the kind and extent of weathering that it may be used, perhaps, in the classification of soils. For example, in figures 2a and 2b there appear to be two types of acids represented by four different soils. The nature of the acid in soils 675—Norfolk sandy loam—and 680—Greenville fine sandy loam—is the same, even though the Norfolk has weathered from unconsolidated sandstone and the Greenville from limestone. They are both Coastal Plain soils and have weathered to about the same extent. Soil 386—Decatur silt loam—and soil 674—Cecil clay loam—appear to have the same type of acid. The have weathered similarly and represent older soils than the Norfolk and the Greenville. The Decatur has weathered from limestone and the Cecil from silicate rocks. Likewise, in figure 6, the nature of the acid in soil 676—Colby silt loam from

Wisconsin, a soil derived from loessial material—is similar to that in soil 679—Miami silt loam, weathered from glacial till in the late Wisconsin drift. Both soils have weathered to approximately the same extent under the podzolic type of weathering. Kerr (13) has shown that the base exchange complex in these two soils is similar. Furthermore, if the soils of the coastal plain Black Belt are considered (figs. 3 and 4), the nature of the acid appears to be a criterion of the extent of weathering in these soils. These soils are derived from the same geologic deposit, namely, a heavy marine clay deposited on the Selma chalk. The Lufkin is the least weathered, has the highest SiO_2-sesquioxide ratio, and possesses the weakest acid. The Oktibbeha is the most highly weathered, has the lowest ratio, and possesses the strongest acid. This comparison holds true in comparing the surface horizons which are the most highly weathered zones in the profile.

Gedroiz (8) has suggested that the absorbed cations in the soil exchange complex may be used in a genetic classification of soils. He has divided all soils into several categories on this basis. Podzolic and lateritic soils are classified as soils containing exchangeable hydrogen in the exchange complex. The latter are characterized by a strong decomposition of the complex throughout the entire profile (true laterite) with an accumulation of Al_2O_3 and Fe_2O_3. The exchange complex of the former is decomposed only in the surface horizons. The data presented in the foregoing indicate that the nature of the soil acid as determined by an analysis of its titration curve may also serve as a basis for characterizing soils.

Soil acidity as affected by the SiO_2/R_2O_3

An analysis of the data in table 2 and figure 7 clearly shows that there is no one direct relationship between the SiO_2-sesquioxide ratio and the nature of the soil acids. This may seem contradictory to the previous work of Anderson, Mattson (15), and Parker and Pate (18). However, as previously mentioned, the results of these investigators may be interpreted in several ways dependent upon the number and kind of soils that were studied. The result in figure 7 indicate that there may be three groups of soil colloidal materials in which there tends to be a direct relationship between the SiO_2-sesquioxide ratio and the total absorptive capacity of the colloid as well as the relative strength of the soil acids. The data of Parker and Pate (18) show that there may be two such groups in their respective colloids.

A satisfactory explanation for these three "apparent groups" cannot be given because of an insufficient knowledge of the exact chemical nature of the soil exchange complex. Two possibilities, however, seem to be manifested. Following the suggestion that the soil exchange complex may form in any one or all of three ways, and that it can vary according to the kind and extent of weathering, these three "apparent groups" may be the result of different types of exchange complexes. On the other hand, different weathering processes are conducive to the accumulation of various amounts of the free oxides

of Al, Fe, and Si. SiO_2 tends to accumulate under podzolic types of weathering. Lateritic weathering is characterized by an accumulation of Al_2O_3 and Fe_2O_3. If free oxides were present the observed SiO_2-sesquioxide ratio would not represent the true ratio of the exchange complex. Mattson (15) has shown conclusively that the presence of free sesquioxides materially affects the absorptive properties of the soil. Colloidal material from a Norfolk soil that originally had a SiO_2-sesquioxide ratio of 1.63 and a total exchange capacity of 20.7 milliequivalents per 100 gm. of colloid, was found to have a ratio of 2.19 and an exchange capacity of 42.5 milliequivalents after the free sesquioxides had been removed. The results in figure 7 may be explained, therefore, on the bases that some of these colloids contained free Al_2O_3 and Fe_2O_3 and others free SiO_2 Perhaps if the true chemical composition of the exchange complex was determined, the results would show a more direct relationship.

During the process of electrodialysis the free oxides of Al and Fe moved towards the cathode and deposited on the membrane next to the cathode chamber where they encountered an alkaline reaction. This resulted in a discoloration of the membrane. Observations showed that some of these colloidal materials apparently contained no free oxides of Al and Fe because the membrane was not discolored. However, this did not indicate an absence of free SiO_2. It was interesting to note that most of the soils that apparently contained few free sesquioxides fell in the middle class in figure 7.

THE RELIABILITY OF PRESENT METHODS OF ANALYZING THE EXCHANGE COMPLEX

It is very evident from the foregoing discussion that many of the apparent discrepancies in the relation of the SiO_2-sesquioxide ratio to the absorptive properties of the exchange complex and the nature of the soil acids are associated with an incomplete understanding of the true chemical composition of the complex. Present methods for analyzing the exchange complex involve the separation of the colloidal material from the soil and the determinations of the different elements in the fraction thus obtained. Data have been obtained, thereby, that cannot be satisfactorily correlated. If the wide variations in the technique of isolating the so-called exchange complex as well as the possibilities of its containing free oxides are considered, it is apparent that satisfactory relationships between the SiO_2-sesquioxide ratio and the properties of the colloids cannot be expected.

It appears logical, therefore, to suggest that present methods be so modified that the colloidal fraction of soils as isolated by different investigators will be fairly uniform as far as its mechanical composition is concerned. In addition to this, the colloidal material should be analyzed for the free oxides of Al, Fe, and Si. This undoubtedly will require a thorough study of analytical methods. Gedroiz (8) suggests a mild KOH extraction for determining the free SiO_2 in soils. Mattson (15) determines the free sesquioxides by peptization with a hot $AlCl_3$ solution. His preliminary data show some very interesting relationships.

If the free oxides of the soil colloidal material can be accurately determined

without destruction of the exchange complex, the results obtained will undoubtedly not only give a more complete conception of the true nature of the exchange complex as developed under different conditions, but also the relation of the accumulation of free oxides to these same conditions. Until more is known concerning the effect of free oxides, satisfactory relationships between the chemical composition of the soil colloidal material and its physico-chemical properties will not be obtained.

SUMMARY

Colloidal material has been isolated from 21 different soils representing various kinds and states of weathering. The SiO_2-sesquioxide ratio, the total exchange capacity, and the nature of the soil acids have been determined on these colloids.

The following results have been obtained in this study:

The nature of soil acids varies considerably in different soils. In weathered soils their nature is solely a function of the kind and extent of weathering and is independent of the parent material. This indicates that there is more than one type of soil acid.

Colloidal material extracted from soils that are well weathered and that have a high SiO_2-sesquioxide ratio tends to be more highly buffered and exhibits stronger acidity than those colloids having a low ratio. Their total exchange capacity is higher.

The buffer capacity of the colloids appears to be primarily a function of the nature of the soil acid.

The nature of the soil acids may prove to be a valuable criterion in the classification of soils.

The exchange complex may develop by removal of certain constituents from the original alumino-silicate minerals; by mutual flocculation of the colloidal oxides of Al, Fe, and Si; and by the precipitation of Al, Fe, and Si from solution.

There is no one direct relationship between the SiO_2-sesquioxide ratio and the total exchange capacity of the colloid, or the nature of the soil acid. Three different groups appear to be manifested in which a direct relationship between these factors is indicated. It is suggested that this may be due to different types of exchange complexes, or to the presence of free oxides of Al, Fe, and Si.

It is suggested that in order to study more accurately the relation of the chemical composition of soil colloidal material to its physicochemical properties the present methods be so modified that a uniform colloidal fraction is isolated from the soil, and that the free oxides of Al, Fe, and Si are not considered in expressing the SiO_2-sesquioxide ratio.

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A METHOD OF OXIDIZING AND DISSOLVING SOIL FOR THE DETERMINATION OF TOTAL AND FILTERABLE MANGANESE AND PHOSPHORUS[1]

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The following method of preparing soil solutions for the determination of total and filterable manganese and phosphorus has been found to be simpler than the official methods. The colorimetric analyses used enable greater accuracy on small quantities of the elements than the older standard methods.

SOLUTION OF SMALL SAMPLES FOR TOTAL ANALYSES

Put 1 gm. of finely pulverized soil (ground to pass a 50-mesh sieve or finer) into a dry 500-cc. Kjeldahl flask. Add 2 gm. of sodium chlorate. Wash down the sides with 25 cc. of 50 per cent (by volume) H_2SO_4 and gently mix. Adjust the free flame to full height and immediately place it under the flask. This is done so that the air above the solution may be heated more rapidly than the solution and decompose the explosive chlorine peroxide as fast as it is liberated. If excessive green fumes form, care must be taken to prevent an explosion. In an open flask, however, the explosion is seldom serious. If the acid concentration is correct and the heat is applied properly, chlorine peroxide will not accumulate and no explosion will occur.

If total nitrogen is to be estimated, the flask may be connected to a distillation apparatus as described in an earlier article (3). If violent reaction begins withdraw the flame until violence ceases and then continue the heating carefully. When green fumes cease forming and the action is no longer violent, heat vigorously until the solution is clear, water has been driven off, sulfur trioxide fumes form, and sulfuric acid condenses on the sides. A green color of the solution at this point indicates manganese and proves that oxidation is complete. If the residue and solution are not free of organic matter add more chlorate in small portions at a time until the solution clears. When organic matter has been oxidized, chlorine driven off, and the solution cooled somewhat, add 75 cc. of water and shake well. If there is much residue the solution should be boiled until it is certain that all material that will dissolve has gone into solution. Make up to exactly 100 cc. The residue should be colorless and translucent if

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

all minerals have been dissolved and only siliceous material remains. If the residue settles readily, aliquots of the clear solution may be pipetted off for analysis. Filter, if necessary, and use appropriate aliquots for estimation of total manganese and phosphorus.

SOLUTION OF LARGE SAMPLES FOR TOTAL ANALYSES

If more than 1 gm. of soil is used, add 5 cc. of concentrated nitric acid and 1 to 2 gm. of chlorate for each gram of sample. Heat with a high free flame. The oxidation is much less violent than in sulfuric acid and the heating should be continuously strong, unless foaming becomes excessive. Little chlorine peroxide is formed when a high strong flame is used. Heat until the residue is dry. Cool, add about 0.1 gm. of chlorate and 5 cc. of 50 per cent (by volume) sulfuric acid for each gram of soil. Heat and expel chlorine as with smaller samples. Take up the carbon-free salts as before.

TOTAL MANGANESE

To an aliquot containing 1 to 5 mgm. of manganese add concentrated sulfuric acid to make the amount of this acid in the solution about 20 cc. Add 20 cc. of nitric acid. Make up to about 70 cc. and analyze for Mn by the official method (1) with modifications as made by Willard and Greathouse (10), as follows: Add 0.05–0.1 gm. potassium periodate and boil until a permanganate color develops. Boil one minute and add a slight excess of periodate. Keep hot 10 minutes, then make to a known volume and match with a standard. A concentration of 0.01 to 0.05 mgm. of Mn per cubic centimeter is most satisfactory for readings in a colorimeter. The standard should be made as directed by Willard and Greathouse (10), or according to the official method (1).

TOTAL PHOSPHORUS

Make an aliquot containing 0.1 to 0.5 mgm. phosphorus up to about 50 cc. and add a few drops of phenolphthalein. Add 10 per cent NaOH drop by drop until a pink color appears. Make up to 70 cc. and determine phosphorus by the method of Fiske and Subbarow (6), making a few minor changes as follows: Add 10 cc. of 2.5 per cent ammonium molybdate made up in 5 *N* sulfuric acid. Mix well and add 4 cc. of 0.25 per cent 1,2,4-aminonaphtholsulfonic acid prepared as directed by Fiske and Subbarow (6). At the same time treat a standard solution in the same manner. (Dissolve 0.1755 gm. potassium dihydrogen phosphate in 1,000 cc. of water. Treat 5 cc. of this stock solution containing 0.2 mgm. phosphorus as described in the foregoing, and dilute to 100 cc., making a solution containing 0.002 mgm. of phosphorus per cubic centimeter.) Make up the unknowns to 100 cc. and after 10 minutes compare in a colorimeter. Calculate the results to per cent of total phosphorus in the sample. The method of Deniges as used by Truog and Meyer (9) may be applied to this solution, although the foregoing method is preferred because of certain advantages (5).

RESULTS ON TOTAL MANGANESE AND PHOSPHORUS IN SOIL

Karraker[2] (table 1) used the foregoing method for destroying organic matter and dissolving manganese, and obtained more manganese from two out of three soils than Shedd did by sodium carbonate fusion (8). Karraker (7) used the chlorate method in determining manganese in his study of the effect of manganese on the quinhydrone electrode.

In order to test for manganese which might be held in the insoluble silicates, the thoroughly washed white silica residues were fused with sodium carbonate and tested for manganese by the periodate method. No traces could be de-

TABLE 1

Comparison of Mn found by chlorate and by sodium carbonate fusion

SAMPLE	CHLORATE BY KARRAKER, PER CENT OF AIR-DRY SOIL	Na_2CO_3 FUSION BY SHEDD, PER CENT OF AIR-DRY SOIL
36552	0.077 0.075	0.050
56496	0.119 0.125	0.136
14614	0.240 0.250	0.198

TABLE 2

Total manganese and phosphorus in soils

SOIL	Mn			P		
	Determination 1	Determination 2	Error from average	Determination 1	Determination 2	Error from average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	0.400	0.420	2.4	.....	.....	.....
2	0.049	0.045	4.2	.....	.....	.....
3	0.188	0.200	3.1	.....	.....	.....
4	0.300	0.312	2.0	0.222	0.222	0
5	0.180	0.170	2.9	0.091	0.091	0

tected. When the residues from three determinations were combined and fused with sodium carbonate, however, a faint trace of manganese did appear. This might, of course, have been from other sources than the insoluble silica.

Phosphorus gave almost perfect checks in all cases tried. Table 2 gives results typical of those obtained in the analyses for total manganese and phosphorus.

In order to determine whether silica might cause the phosphorus readings to be too high, as noted by Truog and Meyer (9), two 1-gm. samples of pure silica

[2] P. E. Karraker, Ky. Agri. Exp. Sta., unpublished data.

were treated as the samples were treated in the foregoing procedures, and the solution was tested for phosphorus. No blue color developed. This indicates that not enough silica was brought into solution to cause error in the test. Evidently silicic acid is dehydrated by the evaporation to dryness and sulfuric acid treatments.

For comparison, phosphorus was determined in samples of soil that had been analyzed by S. D. Averitt,[3] in the routine work of the soil survey, by the official magnesium nitrate method.

Table 3 presents five comparisons between the two methods. In every case the chlorate method gave slightly larger amounts of total phosphorus. In soils 1 and 5, containing about 0.06 per cent phosphorus, the chlorate method gave 0.007 per cent more total phosphorus than the magnesium nitrate method; in soil 4, containing abnormally large amounts of phosphorus, 0.222 per cent more phosphorus was found; and in soil 2, which is low in phosphorus, only 0.001 per cent more phosphorus was found.

TABLE 3

Comparison of total phosphorus in soils by the official magnesium nitrate method and by the chlorate method

Phosphorus as per cent of air-dry soil

SOIL NUMBER	CHLORATE METHOD			MAGNESIUM NITRATE METHOD	DIFFERENCE
	Determination 1	Determination 2	Average		
1	0.063	0.061	0.062	0.055	+0.007
2	0.033	0.033	0.033	0.032	+0.001
3	0.156	0.150	0.153	0.126	+0.027
4	1.020	1.088	1.054	0.832	+0.222
5	0.068	0.065	0.067	0.060	+0.007

FILTERABLE MANGANESE AND PHOSPHORUS

As yet there is no standard method for the determination of minerals available to plants. One investigator recommends treating the soil with 0.2 *N* nitric acid; another, with 0.1 *N* hydrochloric acid; and, a third, with 0.01 *N* hydrochloric acid. There are others who use ammonium chloride and citric acid as solvents. In an experiment on the effect of soil reaction on plant growth it was found that existing methods for determining the availability of minerals were not applicable, because the addition of nearly all the solvents brought all soils to approximately the same reaction and masked the effect of the varying degree of acidity.

Comber (2) states that there are three possible ways for plants to obtain soil nutrients; namely, by absorbing minerals naturally in true solution, by dissolving readily available minerals through root action, and by absorbing minute

[3] Of the chemistry department of the Agricultural Experiment Station, University of Kentucky.

colloidal particles. It is apparent that minerals in true solution or those in a fine state of colloidal division are the ones most likely to be absorbed by plants. In order to obtain comparable results as to availability of minerals in the same soil regulated to varying degrees of acidity and alkalinity by various treatments, it was decided to shake soil with water and filter the supernatant liquid through a certain grade of filter paper. The minerals present in this filtrate, including those in solution and those in a very fine state of colloidal suspension, were considered available to the plant. Prolonged shaking with a large quantity of water was avoided, since this would change the degree of acidity by dilution and bring about marked changes in the actual state of the soil. Ten minutes of shaking, allowing the material to stand a few minutes, and immediate filtering of the supernatant liquid should more nearly extract only those minerals in actual solution or in a fine colloidal state in the normal soil solution. This treatment should break up the soil films.

It is likely that the various analytical solvents and plant roots concentrate their action largely on the fine colloidal particles, and consequently a total analysis of these particles along with the minerals in actual solution should give a very close approximation to the available minerals in the soil without introducing foreign elements such as the hydrogen ion, the nitrate ion, the chloride ion, and the citrate ion.

The procedure adopted and a few of the results obtained follow.

Procedure used in determining filterable manganese and phosphorus in a uniform soil adjusted to varying hydrogen-ion concentrations

Place 100 gm. of air-dried soil in a 500-cc. Erlenmeyer or shaker bottle and add 2 cc. of distilled water for each gram of soil. Shake vigorously for 10 minutes. Allow to settle several minutes and decant upon a no. 2 genuine Whatman filter paper. Allow as little of the settled soil to go on the filter as possible. Pour back the first 10 to 20 cc. of filtrate. Evaporate a suitable aliquot of the filtrate to a small volume, taking care to keep any colloidal material well distributed by shaking before measuring. Treat the residue exactly as in the analysis of soil for total manganese and phosphorus. The amount of chlorate and the kind and amount of acid used should be regulated by the amount of organic matter to be destroyed. Usually only small amounts of chlorate and sulfuric acid are necessary.

Results on filterable manganese and phosphorus in soils

Tables 4, 5, and 6 present data which were obtained primarily to ascertain the effect of reaction on the availability of phosphorus and manganese in the soil. The tables are presented here only to verify the method used and the discussion will be limited to a comparison of duplicate treatments and samples.

Table 4 shows the amounts of filterable manganese found in plots treated with similar kinds and amounts of chemicals, but of different position in the house and to which manure was added, as indicated. There is great variation in

a few cases, but when it is considered that the samples were taken from different plots and not from the same composite sample, it seems that the variation is not excessive.

TABLE 4

Filterable manganese found in tomato soils

TREATMENT NUMBER	CHEMICALS ADDED TO PLOTS 1 AND 2		Mn		ERROR FROM AVERAGE	AVERAGE pH OBTAINED	MANURE ADDED TO PLOTS
	Kind	Tons an acre	Plot 1	Plot 2			
			p.p.m.	*p.p.m.*	*per cent*		
1	Check		0.50	0.50	0	7.04	1 and 2
2	$Ca(OH)_2$	5.5	0.25	0.28	5.7	8.52	2
3	H_3PO_4	1.0	0.25	0.56	38.3	6.42	2
4	Na_2CO_3	2.4	9.80	10.00	1.0	8.43	2
5	Na_2CO_3	0.5	2.00	2.03	0.7	7.58	2
6	HNO_3	2.4	3.10	3.10	0	5.85	1 and 2
7	Check		1.33	3.30	42.5	7.09	None
8	H_3PO_4	6.0	3.50	4.25	16.1	5.25	2
9	Check		0.49	0.49	0	6.86	None
10	Na_2CO_3	0.5	1.95	1.95	0	7.70	2
11	$CaCO_3$	18.0	0.12	0.12	0	8.00	2
12	H_2SO_4	2.5	6.22	8.44	15.1	6.15	2

TABLE 5

Filterable phosphorus found in tomato soils

TREATMENT NUMBER	CHEMICALS ADDED TO PLOTS 1 AND 2		Mn		ERROR FROM AVERAGE	AVERAGE pH OBTAINED	MANURE ADDED TO PLOTS
	Kind	Tons an acre	Plot 1	Plot 2			
			p.p.m.	*p.p.m.*	*per cent*		
1	Check		20.0	20.0	0	7.04	1 and 2
2	$Ca(OH)_2$	5.5	11.2	10.7	2.3	8.52	2
3	H_3PO_4	1.0	57.6	51.2	5.9	6.42	2
4	Na_2CO_3	2.4	98.3	89.1	4.9	8.43	2
5	Na_2CO_3	0.5	79.9	83.1	1.9	7.58	2
6	HNO_3	2.4	23.2	24.0	1.7	5.85	1 and 2
7	Check		8.2	8.6	2.4	7.09	None
8	H_3PO_4	6.0	192.0	200.0	2.0	5.25	2
9	Check		19.2	24.8	12.7	6.86	None
10	Na_2CO_3	0.5	27.2	33.6	10.5	7.70	2
11	$CaCO_3$	18.0	24.0	24.0	0	8.00	2
12	H_2SO_4	0.5	9.1	8.3	4.6	6.15	2
13	HNO_3	1.0	11.5	12.2	2.9	6.74	2
14	H_3PO_4	5.0	280.0	280.0	0	5.10	None
15	$Ca(OH)_2$	5.5	5.3	5.6	2.7	8.70	2
16	$CaCO_3$	18.0	8.0	8.3	1.8	8.00	2
17	H_2SO_4	2.5	28.3	21.7	13.2	5.61	2
18	H_2SO_4	3.0	26.4	32.0	9.5	5.20	2

Table 5 includes data on filterable phosphorus from the same plots as in table 4 and a few additional cases. The variation from the average here does not exceed 13.2 per cent and as a rule is under 5 per cent, which is good, considering that the duplicates were not run on composited samples from the same plot, but from composited samples from plots of similar treatment.

In order to check the method further, two determinations were made on a composite sample from a single plot. Table 6 shows that the amounts of filterable phosphorus in the two determinations from the same composite sample are practically identical for each treatment or hydrogen-ion concentra-

TABLE 6

Filterable phosphorus in soils in which lettuce was grown

Two determinations on composite samples of the same air-dry soil from each plot

PLOT	TREATMENT	TONS AN ACRE	DETERMINATION 1	DETERMINATION 2	ERROR FROM AVERAGE	AVERAGE pH OBTAINED
			p.p.m.	*p.p.m.*	*per cent*	
1	New soil check		11.20	.....	...	5.80
2	Old soil check		8.64	8.64	0	6.86
3	$Ca(OH)_2$	6.6	7.04	6.40	4.7	8.34
4	$CaCO_3$	17.6	7.36	7.36	0	7.84
5	$CaCO_3$	3.3	5.76	5.76	0	7.31
6	H_2SO_4	1.4	8.96	8.32	3.7	6.14
7	H_2SO_4	2.6	22.40	22.40	0	5.61
8	H_2SO_4	5.9	25.60	25.60	0	4.64
9	HNO_3	2.9	10.24	10.24	0	5.98
10	Old soil check		8.64	8.64	0	6.90
11	Na_2CO_3	3.1	27.60	27.60	0	8.76
12	Na_2CO_3	1.0	11.56	11.56	0	7.70
13	Na_2CO_3	0.5	8.96	8.64	1.8	7.31
14	H_3PO_4	1.4	48.00	48.00	0	6.23
15	H_3PO_4	3.5	115.20	115.20	0	5.75
16	Old soil check		8.64	7.68	5.9	6.85
17	H_3PO_4	8.1	264.00	.....	...	5.24
18	Old soil check		9.28	9.60	1.7	6.79
19	HNO_3	0.8	6.40	6.40	0	6.49
20	New soil + Na_2CO_3	0.33	11.20	10.88	1.4	6.84

tion which existed in these soils. Table 6 also shows duplication between like treatments. For instance plots 2, 10, 16, and 18 are check plots and contain almost identical amounts of filterable phosphorus.

SUMMARY

Soils are prepared for total manganese and phosphorus analyses by destroying the organic matter and bringing the minerals into solution by heating with sulfuric acid and sodium chlorate.

Findings for manganese and phosphorus are presented.

The procedure is simpler than the usual methods and the danger of loss of the elements may be less.

A method for determining relative availability of manganese and phosphorus without introducing foreign elements is proposed. The soil is shaken for 10 minutes with water allowed to stand a few minutes, and the supernatant liquid filtered through a genuine Whatman no. 2 filter paper. The filtrate, which may include colloidal particles as well as substances in solution, is assumed to contain the approximate amount of manganese and phosphorus compounds that are available as plant-food. Manganese and phosphorus are determinated in this liquid in nearly the same way as described for total analysis.

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HYDROGEN-ION CONCENTRATION, ALUMINUM CONCENTRATION IN THE SOIL SOLUTION, AND PERCENTAGE BASE SATURATION AS FACTORS AFFECTING PLANT GROWTH ON ACID SOILS[1]

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Much investigation of soil acidity in its relation to plant growth has resulted in the proposal of a number of theories for explaining the data obtained. Although it is realized that soil acidity may affect plant growth in a variety of ways, as has been pointed out by Truog (44), the question as to which factors of acidity are directly concerned and are of primary importance remains to be determined. Robinson (37) recently expressed this viewpoint as follows: "In so-called acid soils we have a combination of acid reaction, active aluminum, low calcium content, and other factors all more or less correlated among themselves. It is important to know which of these factors is the primary factor of which the others are only secondary consequences."

Since the introduction of the hydrogen electrode into soil investigations, much has been claimed for the value of determinations of hydrogen-ion concentration in explaining the relationship between soil acidity and plant growth (3, 24, 39, 40, 46). Various lines of evidence suggest, however, that the hydrogen-ion concentration of soils cannot be considered a direct or primary factor in causing poor plant growth on acid soils. In the first place, conflicting results have been obtained by various investigators (7, 26, 36, 42) regarding the optimum reaction for the growth of the same crops. Secondly, several investigators (12, 13) have reported little correlation between hydrogen-ion concentration of soils and the response of crops to liming.

Both Duley (12) and Fleetwood (13) conclude from their works that the amount of available soil calcium as determined by extraction with carbonated water, is a better indication of the response of plants to liming than is the total acidity of the soil or its hydrogen-ion concentration.

On the other hand, much evidence has been submitted in favor of the toxic aluminum theory of soil acidity by a number of American investigators (4, 8, 10, 22). Without going into any detail regarding the investigations upon which this theory is based, the recent criticism of the theory by Line (17) raises some question as to its validity.

[1] Published with the approval of the director of the Alabama Agricultural Experiment Station. Part of the work connected with this paper has been done since the writer has been with the West Virginia Agricultural Experiment Station.

Moreover, the recent development of the base exchange conception of soil acidity suggests that plant growth on acid soils may be more closely related to the condition of the soil with respect to base saturation than it is to hydrogen-ion concentration or aluminum toxicity.

This investigation was started with the following objectives:

To compare different soils with respect to the hydrogen-ion concentration at which plant growth is definitely reduced ("critical hydrogen-ion concentration").

To determine the concentration of aluminum and other elements in the displaced soil solution at various degrees of acidity and to study their effect on plant growth.

To study the relationship between plant growth on acid soils and the ratio of potassium to calcium in the displaced soil solution.

To study the influence of the percentage base saturation of acid soils on plant growth.

EXPERIMENTAL METHODS AND PROCEDURE

Thirteen large samples of soils were obtained, seven from Alabama and the remainder from four other states.[2] They were selected with the purpose of having soils formed under different climatic conditions and which were of different degrees of weathering. A description of the soils is given in table 1. Each soil was thoroughly mixed and passed through a one-fourth inch screen upon being taken to the greenhouse. Seven kilograms of dry soil was then placed in each of ten 2-gallon glazed earthenware pots. This provided two pots for each of the five different soil treatments to be given.

Treatment of soils

The different treatments consisted of bringing each soil to five different degrees of reaction. This was done by additions of lime or of a combination of nitric and sulfuric acids. In all cases but one the soils had original reactions between pH 5.20 and 6.00. Three of the treatments consisted of additions of a combination of equal amounts of normal nitric and sulfuric acids to bring the soils to pH values of 4.80 to 5.10. These acids are believed to simulate the action of ammonium sulfate on soils. The other treatment consisted of liming each soil according to its respective content of exchangeable hydrogen, determined according to the barium acetate method (27). The other set of pots received no lime nor acid treatments. In the case of soil 671, all were lime treatments, since the soil had an original pH value of 4.40. The amount of acid required to bring the soils to the pH values desired was determined by the buffer method previously described (31). The acid was added by dumping the soil from a pot into a large pan and by adding the acid in a fine spray while mixing the soil in the pan. The lime was added in the form of precipitated calcium carbonate. After the soils were put back into the pots, rain water was added to bring them to approximately optimum moisture. They were kept

[2] The writer wishes to express his appreciation to the following men for furnishing soil samples from their respective states: Prof. W. E. Ayres, Mississippi; Dr. F. C. Bauer, Illinois; Dr. T. S. Buie, South Carolina; Prof. A. R. Whitson, Wisconsin.

at about this moisture content for six months by further additions of rain water when necessary. At the end of two and four months the soil in each pot was mixed thoroughly and a sample taken for determination of hydrogen-ion concentration. At the end of six months the soils were leached with rain water,

TABLE 1

Description of soils used in this investigation

SOIL NUMBER	SOIL TYPE	GEOLOGIC CLASSIFICATION OF SOILS	H-ION CONCENTRATION	ORGANIC MATTER (ORGANIC C × 1.724)	TOTAL EXCHANGE CAPACITY	$\frac{\text{mols } SiO_2}{\text{mols } R_2O_3}$ OF COLLOIDS
			pH	*per cent*	*mgm. equiv.*	
670	Grundy silt loam (Ill.)	Derived from Peorian loess under conditions of poor drainage	5.20	6.07	29.37	2.99
671	Cory silt loam (Ill.)	Derived from shallow deposits of Sanganon and Peorian loess on top of Illionian glacial drift	4.58	1.91	8.88	3.20
672	Cecil sandy loam (S. C.)	Residual. Piedmont Plateau	5.88	3.48	7.29	1.78
673	Delta light silt loam (Miss.)	Alluvial, from Miss. River	6.60	1.48	9.47	2.66
674	Cecil clay loam (Ala.)	Residual. Piedmont Plateau	6.15	1.57	5.57	1.57
675	Norfolk sandy loam (Ala.)	Marine. Coastal Plains	5.60	0.97	2.35	1.92
676	Colby silt loam (Wis.)	Glacial and loessial	5.63	3.64	14.54	3.18
678	Cecil clay (Ala.)	Residual. Piedmont Plateau	5.43	1.60	4.35	1.48
679	Miami silt loam (Wis.)	Glacial till	5.85	3.36	10.68	3.97
680	Greenville fine sandy loam (Ala.)	Marine. Coastal Plains	5.40	2.62	8.26	1.62
683	Susquehanna fine sandy loam (Ala.)	Marine. Coastal Plains	5.40	2.47	5.12	2.01
684	Norfolk sandy loam (Ala.)	Marine. Coastal Plains	5.35	1.57	3.22	—
685	Greenville sandy loam (Ala.)	Marine. Coastal Plains	5.48	1.26	2.43	—

to remove the soluble salts which accumulated through the reaction of the acid with the soil, and thus to simulate field conditions as closely as possible. Seven to twelve liters of water was added to each pot for the different soils, the amount of water added to each pot of the same soil being the same. This leaching was

sufficient to remove practically all the nitrates that had been added to many of the pots in the form of nitric acid. After the soil in each pot had dried partially, it was again dumped into a large pan, thoroughly mixed, and a sample taken for determination of hydrogen-ion concentration. The soils were then fertilized, as described elsewhere in this paper.

Soils 683, 684, and 685 were added to the study later; and sorghum, the second crop grown on the other soils, was the first crop grown on these soils.

Crops grown

The crops grown, in the order of their succession, were corn, sorghum, and barley. Barley and sorghum are known to be sensitive to soil acidity, whereas corn is usually considered fairly acid resistant. The corn was planted May 26, 1928, and harvested July 4. Sorghum was planted August 1, 1928, and harvested when the crop was mature, October 4. Barley was planted November 24, 1928, and harvested in May, 1929. With corn and sorghum three stalks were grown in each pot, whereas with barley five plants were grown in each pot.

Fertilizer treatment of the soils

It seemed best in this study to fertilize all the soils with phosphorus, nitrogen, and potash so that they might be removed as limiting factors and also that any indirect effects of soil reaction on the availability of these elements might be decreased. Before the planting of each crop, the soils were thoroughly mixed with the fertilizer. The phosphate fertilizer was applied for each crop at the rate of 160 pounds of P_2O_5 for each acre of 2,000,000 pounds of soil. For the corn crop this was in the form of superphosphate; for the other two crops monocalcium phosphate was used as the source of phosphorus. The potassium fertilizer was used in the form of potassium nitrate at the rate of 100 pounds an acre for corn and barley, and 135 pounds an acre for sorghum.

In order to prevent the accumulation of large amounts of soluble salts, most of the nitrogen fertilizer was applied at various times during the growing period rather than all at planting time. The amounts and kinds of nitrogen fertilizers applied to each crop are given in table 2. In order to maintain the soils at the desired reactions, a combination of nitrogenous fertilizers that should have no effect on the soil reaction was used (32). This was a combination of physiologically basic nitrogen fertilizer with an acidic fertilizer, ammonium nitrate, in the proportion of two parts of nitrogen in the former and three parts in the latter. Since after the calculated acid treatment, a few of the soils did not have pH values as low as was desired, these received a greater proportion of their nitrogen in the form of acidic sources, as is shown in table 2. The soils upon which plants showed very poor growth or decided acid injury did not receive the additional nitrogen fertilizers applied during the growing period. For the sorghum crop these were pots 3 and 4 of series 672 and 678, pots 3 to 6 inclusive

of soil 684, and pots 3 to 8 inclusive of soil 675. For barley the following pots received only one-third as much nitrogen after planting as is indicated in table 2: pots 3 and 4 of soils 672, 675, 678, and 680; and pots 5 and 6 of soil 675. This was thought necessary in order to prevent the accumulation of excess soluble salts which would be likely to accentuate the injury to the plants.

TABLE 2

The amounts and forms of nitrogen applied for the various crops

(In pounds for each 2,000,000 pounds of soil)

SOILS NUMBER	AMOUNTS OF NITROGEN APPLIED IN DIFFERENT FORMS						
	At planting		During growing period				Total
	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	$NaNO_3$	NH_4NO_3	$(NH_4)_2SO_4$	
For corn							
All but Nos. 670, 673, and 679	13.8		36.2		50		100
Nos. 670, 673, and 679	13.8		11.2		75	40	140
For sorghum							
All except Nos. 670, 673, 676, and 679	18.7	28.0		33.3	50.0		130
Nos. 670, 673, 676, and 679	18.7	71.3				40.0	130
For barley							
All soils	13.8	20.7		36.0	54.0		125

Soil studies

Soil studies consisted of the following: determination of the hydrogen-ion concentration of the soil from each pot taken before and after harvesting each crop; determination of aluminum, phosphorus, calcium, potassium, and manganese in the displaced soil solution from each pot obtained after the growth of corn and before fertilizing for sorghum; and determinations of the percentage base saturation of the soils sampled after the growth of corn and before displacing the soil solution. The soil solutions were obtained by the displacement method (27). For this purpose about 2,000 gm. of soil was used from each pot. After displacement, the soils were put back into their respective pots, allowed to dry partially, and then mixed thoroughly with the soil left in the pots.

Chemical methods

The hydrogen-ion concentration of the soils was determined by the "dialysis colorimetric method" (34). When the necessary precautions were taken, this method was shown to give accurate results (33).

The percentage base saturation values of the soils were based on the determinations of exchangeable hydrogen according to Parker's (28) barium acetate method and on the determinations of total exchange capacity according to the $Ba(C_2H_3O_2)_2$-NH_4Cl method (28).

The phosphate content of the soil solution was determined by the blue colorimetric method (29); potassium by the cobalt nitrite colorimetric method as modified by Tidmore (41); and calcium and manganese were determined by the standard methods.

The aluminum content of the soil solution was determined by the "aluminon" method recently proposed by Yoe and Hill (47). After a thorough examination of the method and the adoption of a few slight changes, it was found to be satisfactory. The changes included: the addition of 2 cc. of a 20 per cent ammonium chloride solution before precipitating the iron and aluminum in order to make the separation more complete, the use of a series of standards in which the color is developed at the same time as in the unknown solution, the taking of readings between 20 and 30 minutes after developing the color. It was found that if a longer time than this is allowed, the color in the unknown solutions which have been subjected to the procedure for removal of the iron, fades more quickly than does that of the standards. The recovery of aluminum when added in concentrations of 0.5, 1.0, and 2.0 p.p.m. averaged about 97 per cent. Iron, if not precipitated, was found to interfere even when present in concentrations of only one-fourth that of aluminum. However, by removal of the iron, according to Yoe and Hill, good recoveries of aluminum were obtained even when iron was originally present in equal concentrations. Although most of the soil solutions analyzed apparently contained very little iron, as indicated by the color of the precipitate, the procedure for removal of iron was employed in all cases. A comparison of the "aluminon" method with the gravimetric method of Lundell and Knowles (19) gave the following results on an acid soil extract: "Aluminon method," 3.2 p.p.m.; gravimetric method, 2.9 p.p.m.

EXPERIMENTAL DATA

In the presentation of these data a brief description of the characteristic plant injury noted from soil acidity is first given. The data for each of the various factors of soil acidity in its relation to plant growth are then presented in the following order: hydrogen-ion concentration, aluminum toxicity, potassium-calcium ratio in the soil solution, and percentage base saturation. As far as possible, the various factors will be considered in relation to one another. For this purpose a summary table (table 6) was prepared, to which references are made throughout the paper.

In this study the yields of sorghum are no doubt the most valuable in indicating the effect of the various factors of soil acidity on plant growth, for more soils can be compared, and the depressions in yields are much more pronounced than with corn. The yields of barley were also markedly depressed by acidity, but since the determination upon which the data in table 6 are based were made

on the soil before the sorghum crop was planted, some of these values may not hold very strictly for the soil at the time the barley crop was growing. Moreover, the barley crop did not make as vigorous and normal growth as did the sorghum. These facts should be kept in mind in the following discussion.

TABLE 3

Notes on the early growth of sorghum as affected by soil acidity

SOIL NUMBER	INJURY TO SEEDLINGS 1 WEEK OLD (BY POTS)	RELATIVE GROWTH, BASED ON 100, FOR PLANTS IN POTS 9 AND 10 (PLANTS 23 DAYS OLD)				NOTES ON APPEARANCE AND EXTENT OF INJURY WHEN PLANTS WERE 23 DAYS OLD
		Pots 1–2	Pots 3–4	Pots 5–6	Pots 7–8	
		*	*	*		
671	1, 2, 3, 4	15	25	30		Plants in pots 1 and 2 are chlorotic but less so than some of the plants in soils 672, 674, and 675. A slight blotching with reddish brown spots on leaves in pots 3 and 4
672	3, 4	100	20	40	100	Plants in pots 3 and 4 are very chlorotic. Yellow stripes along veins of leaves, with some reddish brown mottling. Leaves of these plants have wilted appearance. Plants in pots 5 and 6 less injured than those in pots 3 and 4
674	3, 4	100	40	90	100	Leaves of plants in pots 3 and 4 are chlorotic, striped, and mottled as are those described from soil 672. Some mottling in plants of pots 5 and 6
675	3, 4, 5, 6	100	5	5	20	Plants in pots 3 to 6 are very chlorotic. Leaves are turning brown at tips, otherwise similar to plants in soil 672. Plants in pots 7 and 8 slightly chlorotic
678	3, 4	100	20	100	100	Plants in pots 3 and 4 have pale green or mottled appearance, but are not wilted in appearance or mottled as are those of plants in soil 672, 674, and 675
680	3, 4	100	90	100	100	Plants in pots 3 and 4 are slightly chlorotic, striped, and blotched in appearance along the edges of the leaves
684		100	50	60	100	Plants in pots 3 to 6, although chlorotic, do not show the mottled appearance as do those in soils 672, etc.
685		100	30	100	100	Plants in pots 3 and 4 are beginning to show distinct yellowing and blotching

Plants in pots 3, 4, 5, and 6 of soil 679 showed injury when 1 week old, but this injury was overcome later. The plants grown in all the other soils not included in this table did not show injury and were apparently making good growth (see also tables 4, 5, and 6).

* Based on pots 7 and 8 as 100.

Characteristics of the plant injury from soil acidity

Plant injury on acid soils is indicated not only by the total yields obtained but also by the appearance of the plants at early stages of growth. In these experiments, the plants grown on some of the acid soils first showed injury from acidity not in growth, but in the appearance of the leaves. With corn the characteristic injury to the seedlings was noted when they were less than 1 week old. The plants were just as tall as those grown on the less acid soils, but there was present a yellowing of the leaves in stripes between the veins. In extreme cases of injury a large part of the leaf had this light yellow color. As the plants grew older, the injury to the leaves disappeared and the new leaves were normal except in extreme cases of injury. In some cases the striation persisted for several weeks without apparent injury to growth.

Notes were also made of the appearance of the sorghum plants and of their relative growth during the early growing period. A brief summary of these is given in table 3. As in the case of corn, the seedlings grown on some of the most acid soils had the characteristic loss of chlorophyll in stripes between the veins when they were only 5 to 6 days old. The plants showing this abnormal condition are indicated in table 3. During the second week of growth slight differences in the size of the plants began to be apparent. The relative growth of the plants grown on each soil was estimated when they were 23 days old. It will be noted that the relative growth of the plants showing acidity injury, as compared with the plants grown on the limed soils, was in some cases much more than is shown in the final yields. This indicates, as was also observed for the corn and barley crops, that injury to growth in the early stages of growth is sometimes largely overcome in the later stages. Yields at maturity, then, may not always truly represent the extent of the injury. In the field where conditions for growth may be less favorable this early injury would likely have a greater influence on the final yields than under greenhouse conditions.

The acidity injury to barley was also evident in the appearance of the leaves when the plants were very small. The injury was first seen as small yellowish to brown spots and stripes on the leaves especially near their tips. Then the tips of the leaves dried up entirely. As with the other crops, the injury tended to disappear as the plants got older. Whether or not the cause of this early injury is the same as that which caused a reduction in total growth is not known. It is probable that as the plants get older they establish a better root system and are thus able to withstand greater degrees of acidity.

The hydrogen-ion concentration of soils as a factor in plant growth

Various lines of evidence have already been mentioned which suggest that the hydrogen-ion concentration of soils cannot be considered a direct or primary factor in causing poor plant growth on acid soils. In some preliminary studies by the writer in relation to the development of soil acidity by the continued use of acidic nitrogen fertilizers, it was found that one of the soils studied pro-

duced just as good yields of sorghum at pH 4.60 as at any higher pH value; whereas with another soil the sorghum plants showed definite injury and marked reduction in growth as the hydrogen-ion concentration was reduced

TABLE 4

The relation between H-ion concentration of soils and plant growth

SOIL	POT NUMBER	pH VALUE OF SOILS (BEFORE CORN)	YIELDS OF CORN	pH VALUE OF SOIL (BEFORE SORGHUM)	YIELDS OF SORGHUM	pH VALUE of SOILS (BEFORE BARLEY)	YIELDS OF BARLEY	pH VALUE OF SOILS (AFTER BARLEY)
			gm.		*gm.*		*gm.*	
670 Grundy silt loam	1–2	5.20	18.7	5.20	52.8	5.00	20.8	5.03
	3–4	4.83	25.9	4.75	62.8	4.60	20.1	4.55
	5–6	4.98	23.9	4.85	62.0	4.73	17.6	4.75
	7–8	5.08	21.3	4.98	61.0	4.83	20.1	4.78
	9–10	6.60	17.6	6.40	55.6	6.03	21.5	5.98
671 Cory silt loam	1–2	4.58	11.1	4.43	10.0	4.43	12.6	4.43
	3–4	4.63	9.6	4.60	25.6	4.60	17.0	4.55
	5–6	4.90	17.5	4.85	31.6	4.80	18.3	4.78
	7–8	6.18	19.4	6.00	46.6	6.00	21.6	5.88
672 Cecil sandy loam	1–2	5.88	17.9	5.60	29.2	5.78	17.7	5.75
	3–4	4.80	14.3	4.80	5.2	4.80	6.5	4.78
	5–6	5.13	16.9	4.93	23.9	5.08	13.4	5.03
	7–8	5.25	16.8	5.08	32.4	5.30	25.5	5.10
	9–10	6.63	15.7	6.50	30.6	6.35	18.8	6.15
673 Delta light silt loam	1–2	6.60	25.2	5.80	50.9	5.65	25.2	5.78
	3–4	5.00	30.4	4.60	49.3	4.60	22.5	4.80
	5–6	5.23	33.1	4.78	51.7	4.75	20.4	4.88
	7–8	5.50	35.0	4.85	49.7	4.83	20.7	4.98
	9–10	6.83	27.0	6.55	48.7	5.95	19.3	6.00
674 Cecil clay loam	1–2	6.15	13.5	5.80	27.9	5.83	17.8	5.83
	3–4	4.73	11.6	4.75	19.2	4.80	7.1	4.83
	5–6	4.83	14.3	4.85	26.3	4.95	10.0	4.93
	7–8	4.98	15.7	4.95	32.1	5.05	16.8	5.05
	9–10	6.80	13.3	6.78	23.1	6.60	19.0	6.63
675 Norfolk sandy loam	1–2	5.60	12.9	5.40	20.6	5.60	9.1	5.63
	3–4	4.73	5.1	4.75	0.4	4.75	1.3	5.05
	5–6	4.90	8.6	4.93	0.7	4.90	1.1	5.10
	7–8	5.05	11.4	5.03	6.8	5.18	11.9	5.15
	9–10	6.58	13.5	6.43	26.9	6.20	14.2	6.10
676 Colby silt loam	1–2	5.63	20.0	5.53	50.5	5.43	20.6	5.45
	3–4	4.70	15.5	4.80	41.3	4.60	9.8	4.63
	5–6	5.00	17.6	4.95	39.1	4.80	15.8	4.78
	7–8	5.15	14.2	5.05	47.4	4.95	20.2	4.95
	9–10	6.68	20.3	6.58	52.3	6.28	21.4	6.28

TABLE 4—*Concluded*

SOIL	POT NUMBER	pH VALUE OF SOILS (BEFORE CORN)	YIELDS OF CORN	pH VALUE OF SOIL (BEFORE SORGHUM)	YIELDS OF SORGHUM	pH VALUE OF SOILS (BEFORE BARLEY)	YIELDS OF BARLEY	pH VALUE OF SOILS (AFTER BARLEY)
			gm.		*gm.*		*gm.*	
678 Cecil clay	1–2	5.43	11.0	5.38	23.3	5.38	18.9	5.38
	3–4	4.58	3.4	4.45	0.7	4.43	3.4	4.50
	5–6	4.75	9.7	4.80	19.8	4.63	15.9	4.58
	7–8	4.88	11.3	4.93	22.8	4.98	20.4	4.80
	9–10	6.60	9.3	6.50	23.6	6.35	19.7	6.25
679 Miami silt loam	1–2	5.85	29.0	5.40	58.7	5.50	21.7	5.53
	3–4	5.15	29.6	4.93	50.3	4.63	16.3	4.58
	5–6	5.40	30.7	5.03	51.5	4.80	19.6	4.73
	7–8	5.45	26.7	5.03	57.5	4.93	22.5	4.85
	9–10	6.65	29.1	6.45	57.6	6.20	24.2	6.08
680 Greenville fine sandy loam	1–2	5.40	17.3	5.30	30.6	5.40	19.5	5.38
	3–4	4.85	16.2	4.75	26.8	4.65	3.1	4.73
	5–6	4.98	18.7	4.80	27.5	4.78	18.8	4.78
	7–8	5.20	18.1	4.95	27.6	4.88	19.6	5.00
	9–10	6.65	17.9	6.40	34.8	6.35	17.9	6.10
683 Susquehanna fine sandy loam	1–2			5.40	34.6	5.35	19.2	5.40
	3–4			4.80	28.4	4.65	13.5	4.68
	5–6			4.85	28.5	4.88	17.1	4.73
	7–8			5.00	29.9	5.18	21.3	4.90
	9–10			6.20	35.2	6.00	21.3	5.95
684 Norfolk sandy loam	1–2			5.35	17.1	5.80	15.7	5.20
	3–4			4.78	2.1	4.60	13.2	4.70
	5–6			4.83	7.2	4.78	13.3	5.03
	7–8			4.95	17.6	5.40	16.3	5.33
	9–10			6.40	19.1	6.40	15.1	6.40
685 Greenville sandy loam	1–2			5.48	29.3	5.80	15.1	5.68
	3–4			4.95	13.6	4.95	13.5	4.83
	5–6			5.00	22.1	5.20	15.6	5.10
	7–8			5.15	24.0	5.40	16.5	5.10
	9–10			6.48	23.7	6.40	11.6	6.40

to pH 5.0 or below. The data presented in this investigation substantiate these earlier results.

In table 4 are given the yields of corn, sorghum, and barley grown on the different soils each maintained at five degrees of reaction The pH values of the soils before and after the growth of each crop are also given. It may first be noted that corn grew fairly well on many of the soils at low pH values, whereas sorghum and barley showed much greater injury from acidity. Even in the case of corn, however, some differences were obtained in the hydrogen-ion

concentration at which plant growth was materially reduced on the different soils. For example, soil 675, at pH 4.75 to 4.90 produced very poor yields of corn, whereas most of the other soils produced good yields at these pH values; in fact, soils 670, 673, and 679 gave the greatest yields at the most acid reactions.

If the yields of sorghum are considered, it will be noted that the critical hydrogen-ion concentration varies considerably with the different soils. For example, no depression in yields was obtained with soil 670 even at reactions. as low as pH 4.60 to pH 4.75, and practically no depression in yields on soil 673 when as low as pH 4.60; whereas on soil 672 the yield at pH 4.80 was less than 20 per cent of that obtained at higher pH values, and on soil 675 even at pH values slightly above 5.0 the yield was only about 25 per cent of that obtained at pH 6.58. The lack of correlation between hydrogen-ion concentration and plant injury is further shown in table 6. At the uniform pH value of 4.80, it will be noted that the average relative yields of the three crops ranged from 19 to 98 per cent of that obtained from the best culture of the respective series. Similar results are seen to obtain with the growth of barley.

It is thus evident that the hydrogen-ion concentration of soils cannot be considered the controlling factor in determining the growth of plants on acid soils. These data also show the false conclusions that may be drawn when working on a problem of this nature with only a limited number of soils, especially if these soils are of similar origin. If in this study only certain of these soils had been used, the results might have indicated a close correlation between the hydrogen-ion concentration and plant growth. It seems probable that one of the reasons for the good correlation obtained by various investigators between hydrogen-ion concentration and plant growth is that they worked with only few soils or with soils of very similar origin. Under such conditions it is probable that the hydrogen-ion concentration is correlated with or influences other factors which may directly affect growth, (6, 14, 18, 23, 44).

The relation of the concentration of aluminum and other elements in the soil solution to plant growth on acid soils

Many of the investigations on the toxic effect of aluminum on plant growth have been conducted in culture solutions. The limited amount of work carried out with soils has been with relatively few soils, and under such conditions that it has been difficult to isolate the aluminum factor from other factors generally associated with it. If aluminum is the main factor in determining plant injury on acid soils, one would expect to find a relationship between the amounts of aluminum in the displaced soil solution and plant growth. In the fourth column of table 5 are given the data for the concentrations of aluminum in the displaced soil solutions of the various soils at different pH values. It will be noted that the concentrations of aluminum in the soil solutions are in most cases very small even at low pH values. This is in accord with some results by Magistad (23). As was to be expected it was found that the greater the hydrogen-ion concentration of a particular soil, the greater is the concentration of

aluminum in its displaced soil solution. When the different soils are compared, however, it will be observed that the aluminum concentration in their displaced

TABLE 5

The concentration of aluminum and other elements in the soil solution and the percentage base saturation of soils as related to the yield of sorghum

SOIL	POT NUMBER	H-ION CONCENTRATION	CONCENTRATION IN THE DISPLACED SOIL SOLUTION				K/Ca IN SOIL SOLUTION	PERCENTAGE BASE SATURATION	YIELD OF SORGHUM
			Al	PO_4	Ca	K			
		pH	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*			*gm.*
670 Grundy silt loam	1–2	5.20	T	0.68	522	10.4	0.020	63.2	52.8
	3–4	4.75	T	0.60	516	14.6	0.028	56.7	62.8
	5–6	4.85	T	0.56	510	12.8	0.025	58.6	62.0
	7–8	4.98	T	0.72	688	12.8	0.019	60.5	61.0
	9–10	6.40	T	0.52	570	9.1	0.016	89.3	55.6
671 Cory silt loam	1–2	4.43	5.7	0.27	308	26.4	0.086	14.3	10.0
	3–4	4.60	2.7	0.12	616	27.4	0.044	29.1	25.6
	5–6	4.85	0.8	0.17	538	19.6	0.036	32.3	31.6
	7–8	6.00	T	0.24	808	14.2	0.017	73.6	46.6
672 Cecil sandy loam	1–2	5.60	T	0.24	538	29.6	0.055	39.1	29.2
	3–4	4.80	1.1	0.14	444	63.8	0.144	10.0	5.2
	5–6	4.93	0.5	0.17	538	34.2	0.063	18.2	23.9
	7–8	5.08	T	0.20	544	39.2	0.072	22.7	32.4
	9–10	6.50	T	0.24	548	27.4	0.059	69.1	30.6
673 Delta light silt loam	1–2	5.80	T	9.00	782	34.2	0.043	90.4	50.9
	3–4	4.60	1.0	6.80	678	41.0	0.060	71.3	49.3
	5–6	4.78	0.4	8.80	646	38.8	0.060	76.6	51.7
	7–8	4.85	T	9.00	774	39.9	0.051	77.7	49.7
	9–10	6.55	T	8.50	964	32.9	0.034	93.1	48.8
674 Cecil clay loam	1–2	5.80	T	0.07	364	25.1	0.069	51.5	27.9
	3–4	4.75	0.4	0.05	316	38.8	0.122	2.1	19.2
	5–6	4.85	T	0.07	290	31.3	0.108	10.3	26.3
	7–8	4.95	T	0.13	306	24.0	0.078	20.6	32.1
	9–10	6.78	T	0.06	470	21.7	0.046	81.4	23.1
675 Norfolk sandy loam	1–2	5.40	T	1.36	880	68.4	0.078	29.0	20.6
	3–4	4.75	3.7	0.88	706	257.0	0.364	7.1	0.4
	5–6	4.93	1.6	1.02	544	149.4	0.274	12.6	0.7
	7–8	5.03	0.8	1.18	799	86.7	0.108	29.0	6.8
	9–10	6.43	T	1.38	862	68.4	0.079	55.2	26.9
676 Colby silt loam	1–2	5.53	T	0.17	676	13.5	0.020	54.4	50.5
	3–4	4.80	1.1	0.16	424	41.6	0.098	31.5	41.3
	5–6	4.95	0.4	0.16	538	17.3	0.032	35.2	39.1
	7–8	5.05	T	0.18	564	14.4	0.025	39.0	47.4
	9–10	6.58	T	0.15	904	10.9	0.012	78.9	52.3

TABLE 5—*Concluded*

SOIL	POT NUMBER	H-ION CONCENTRATION	CONCENTRATION IN THE DISPLACED SOIL SOLUTION				K/Ca IN SOIL SOLUTION	PERCENTAGE BASE SATURATION	YIELD OF SORGHUM
			Al	PO_4	Ca	K			
		pH	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*			*gm.*
679 Miami silt loam	1–2	5.40	T	1.60	640	22.8	0.035	60.7	58.7
	3–4	4.93	T	1.10	420	51.3	0.124	47.4	50.3
	5–6	5.03	T	1.40	790	27.4	0.034	49.4	51.5
	7–8	5.03	T	1.64	883	21.7	0.024	52.0	57.5
	9–10	6.45	T	1.16	892	16.0	0.018	81.1	57.6
680 Greenville fine sandy loam	1–2	5.30	T	0.31	376	22.8	0.060	26.8	30.6
	3–4	4.75	0.4	0.21	254	29.6	0.116	16.8	26.8
	5–6	4.80	0.5	0.25	388	27.4	0.071	18.2	27.4
	7–8	4.95	T	0.27	388	26.3	0.068	20.4	27.6
	9–10	6.40	T	0.29	568	21.7	0.038	68.5	34.8
683 Susquehnana fine sand	1–2	5.40	T	0.98	280	68.4	0.244	45.7	34.6
	3–4	4.80	2.2	1.00	344	88.4	0.257	28.4	28.4
	5–6	4.85	1.4	1.04	374	85.5	0.228	36.5	28.5
	7–8	5.00	0.9	0.90	380	71.3	0.187	41.5	29.9
	9–10	6.20	T	0.91	470	57.0	0.121	68.6	35.2
684 Norfolk sandy loam	1–2	5.35	T	0.15	368	71.3	0.193	34.3	17.1
	3–4	4.78	1.4	0.10	230	114.0	0.495	17.7	2.1
	5–6	4.83	1.0	0.10	268	88.4	0.329	22.3	7.2
	7–8	4.95	0.8	0.15	372	65.6	0.176	31.0	17.6
	9–10	6.40	T	0.16	468	50.6	0.108	72.0	19.1
685 Greenville sandy loam	1–2	5.48	T	0.44	326	74.1	0.227	57.1	29.3
	3–4	4.95	0.7	0.33	204	125.4	0.614	35.6	13.6
	5–6	5.00	0.9	0.41	246	91.2	0.370	42.9	22.1
	7–8	5.15	0.9	0.41	336	57.0	0.169	49.8	24.0
	9–10	6.48	T	0.44	388	45.6	0.117	82.8	23.7

solutions at similar pH values varied considerably. Soil 670, for example, contained no aluminum in solution even at a pH value as low as 4.75; whereas soil 675 contained 3.7 p.p.m. at this pH value and 0.8 p.p.m. at pH 5.03. At pH 4.60 soil 673 contained 1.0 p.p.m. of aluminum, whereas soil 671 at a similar pH value contained nearly three times this concentration. At the uniform pH value of 4.80 it will be noted (table 6) that the concentration of aluminum in the displaced solution of the various soils ranged from zero with three of the soils to 3.1 p.p.m. with soil 675. These differences can probably be explained, in view of the recent interesting work by Joffe and McLean (15), by differences in the kind of anions present in solution. These investigators found aluminum to be soluble at considerably lower degrees of acidity as the nitrate or chloride than as the sulfate or phosphate.

From the yield data given in table 4, and the aluminum data of table 5, it is

possible to study the relation between the concentration of aluminum in solution and the yields of the various crops. If corn is first considered, it will be observed that some relationship seems to exist. This can also be seen from summary table 6. For example, soil 675, which contained the highest concentration of aluminum at pH 4.80, also showed the greatest injury to plant growth whereas soils 671, 672, and 676, having the next highest concentrations of aluminum, showed the next greatest injury. It will be noted, however, that these last three soils which have the same concentration of aluminum, show considerable difference in the extent of the injury to plant growth. It will also be noted

TABLE 6

Summarized data showing the relation between plant growth at pH 4.80 and percentage base saturation, aluminum concentration, and the K/Ca ratio in the soil solution all calculated for the same reaction

SOIL	Al IN DISPLACED SOIL SOLUTION	K/Ca RATIO IN SOIL SOLUTION	BASE SATURATION OF SOIL	GROWTH OF CORN		GROWTH OF SORGHUM		GROWTH OF BARLEY		AVERAGE* RELATIVE YIELDS FOR THE THREE CROPS
				Injury in early growth	Relative yields	Injury in early growth	Relative yields	Injury in early growth	Relative yields	
	p.p.m.		*per cent*							
670	None	0.027	57	No	100	No	100	No	94	98
671	1.1	0.038	32	Yes	62	Yes	66	No	84	71
672	1.1	0.114	10	Yes	80	Yes	16	Yes	25	40
673	0.4	0.060	77	No	87	No	100	No	83	90
674	None	0.115	6	No	84	Yes	65	Yes	37	62
675	3.1	0.339	9	Yes	47	Yes	2	Yes	9	19
676	1.1	0.098	32	No	79	Slight	76	Yes	74	76
678	Not determined	Not determined	20	No	87	No	89	No	100	92
679	None	0.124	43			Very slight	86	Yes	84	85
680	0.5	0.071	18	No	87	No	79	Yes	96	87
683	2.2	0.257	28			No	81	Yes	80	81
684	1.2	0.354	20			Yes	37	Yes	81	59

* Based on yield of best treatment of each series as 100.

that soil 673 at pH 4.60 showed no depression in yield when it contained 1.0 p.p.m. of aluminum in solution. The soils with little or no aluminum in solution gave very little depression in yield of corn at pH 4.80.

As previously mentioned, the yields of sorghum are probably the most significant ones obtained in this study. With this crop there is less correlation between the amounts of aluminum in solution and the yields. Although in general a correlation seems to exist, there are marked discrepancies. For example, the yield of sorghum in pots 3 and 4 of soil 672 containing 1.1 p.p.m. aluminum was only about 16 per cent of that obtained at pH 6.50, whereas that on soil 673 at

an even lower pH value but with practically the same amount of aluminum in solution was just as good as at pH 6.50. A comparison of the results obtained from soil 675 and 683 is also significant in this respect. These are two sandy coastal plain soils, and both have considerable amounts of aluminum in solution. Soil 675 shows very marked injury to the growth of sorghum with as low as 0.8 p.p.m. aluminum, whereas soil 683 with 2.2 p.p.m. and a more acid reaction gave very little depression in yields as compared with the growth at pH 6.50. Other examples of the lack of correlation between yield of sorghum and the concentration of aluminum in the displaced solution are shown in table 6.

The yields of barley, like those of sorghum, show less correlation with the concentration of aluminum in the displaced solution of these soils, than does corn.

Again it may be pointed out, as was done for the pH data, that if only one or a few of the soils of the same origin are considered, a correlation seems to exist; plant growth, in general, decreases as the amount of aluminum in solution increases.

In order to see whether the aluminum concentration in the soil solution, obtained before the sorghum was planted, remained about constant, the soil solutions of a few of the soils were displaced after removal of the crop and their aluminum concentration was determined. Concentrations of 0.9, 1.3, 1.5, 2.7, and 0.6 p.p.m. aluminum were obtained from cultures 3 and 4 of soils 672, 673, 676, 683, and 685. The corresponding values obtained before sorghum was planted were 1.1, 1.0, 1.1, 2.2, and 0.7. Thus it appears that the concentration of aluminum in the displaced solution of these soils remained quite constant during the growth of the sorghum crop.

The question might be raised as to whether aluminum was present in true solution. Although time did not permit a study of this question it would seem from the results obtained by Magistad (23) and by McGeorge (20) that the aluminum found in the displaced solution of soils is in true solution. No indication of colloidal material could be seen in the soil solutions obtained in this study.

It might also be argued that the concentration of aluminum in the soil solution required to be injurious to the growth of crops depends on other factors such as, for example, the concentration of other ions in solution. Burgess and Pember (8), as a result of their studies, concluded that in the presence of large amounts of phosphate, large amounts of aluminum may be absorbed by plants without affecting the growth. If aluminum is the toxic factor and is rendered less toxic by the presence of phosphate, it might be expected that those soils of the present study with high concentrations of phosphate in the soil solution would show little injury at high degrees of acidity even with considerable aluminum present. As will be seen from table 5, this might explain the fact that a concentration of 1.0 p.p.m. of aluminum failed to be injurious to sorghum on soil 673, for this soil has a very high concentration of phosphorus in its displaced solution. The data for the other soils, however, fail to support this viewpoint.

For example, comparisons of soil 672 with 676 and of soil 675 with 683 show that with similar concentrations of phosphorus and of aluminum in solution, the injury to the growth of sorghum was much more marked on soil 672 than on 676 and also on soil 675 than on 683.

Determinations were also made of the total phosphorus absorbed by the plant, since it seemed probable that the concentration of phosphate in solution did not determine the amount of phosphorus absorbed. Again no relation was found between the percentage of phosphorus in the dry plants and the concentration of aluminum in the soil solutions at which plants showed decreased growth.

Comber (9) has suggested that soil organic matter may be active in reducing the toxic effect of excessive aluminum in the soil solution No evidence of such an action, however, is obtained from the organic matter content of these soils as presented in table 1. It will be noted, for example, that soil 672 has a higher content of organic matter than soil 673, and yet soil 672 produced very decided injury to plant growth when containing 1.1 p.p.m. of aluminum in solution, a concentration at which soil 673 gave no injury.

The investigations of McLean and Gilbert (21) with aluminum in culture solutions are of interest. As a result of their studies they classify corn as resistant, sorghum as mediumly sensitive, and barley as sensitive to aluminum. Olsen (26), also working in culture solutions, found that barley of all the plants studied was the only one to show toxicity from aluminum. It is also significant that the concentrations of aluminum required in culture solution for toxicity to plant growth as found by McLean and Gilbert are considerably greater than those found in the displaced solution of the soils of this study on which plant injury from soil acidity was pronounced. These results suggest that toxicity from aluminum may be different in culture solutions than it is in soils, or that the concentration of aluminum required to be toxic is dependent on other factors. This is an added reason for believing that in most acid soils the main factor is not aluminum toxicity. There was some evidence obtained in this study for the belief that the percentage base saturation of soils is such a factor. This point will be considered in connection with the discussion of percentage base saturation of soils as affecting plant growth.

The displaced soil solutions were also analyzed for manganese. No correlation was obtained between the concentration of manganese in solution and plant injury from acidity. Some of the soils showing the least injury to plants had the highest concentration of soluble manganese.

The ratio of calcium to potassium in the soil solution

Pearsall (30) in his studies of the relation between plant distribution and soil acidity suggested that the ratios of the various cations present in the soil solution may be a more important criterion than is the hydrogen-ion concentration of the soils. Cooper and Wilson (11) have a somewhat similar opinion. Very few data are presented, however, in support of this idea. Salisbury (39) raised an objection to Pearsall's theory, namely, that the ratio of potassium to calcium

is not constant but varies with the soil-water ratio, and he cited results by Burgess to show the differences in the potassium-calcium ratio of soil extracts and soil solutions. In this investigation the calcium and potassium were determined in the soil solution; so his objection would not hold. Salisbury believes, however, that "even if we knew the ratios in the soil solution it appears that the proportion in which the ions are absorbed is not determined by the proportions in which they occur as much as by the reaction of the medium, hence we are again forced back on the pH value of the soil."

In table 5 is shown the potassium-calcium ratio of the soil solutions of the various soils at the different pH values. It is first observed that with nearly every soil the ratio of potassium to calcium decreases as the hydrogen-ion concentration increases. This is in accordance with the results presented by Pearsall from the work of Olsen (25) on soil extracts. Among the different cultures of the same soil it will be observed that there seems to be a relationship between the potassium-calcium ratio and plant growth. The point of interest, however, is not so much the ratios found in the soil solutions of the same soil at different pH values, but the ratios for different soils at similar pH values and the relation of these to plant growth. If such a comparison is made it becomes evident that at similar pH values soils differ considerably in their potassium-calcium ratio. This is brought out in table 6 where the ratios are given for all the soils at pH 4.80. It will be noted that at this pH value the basic ratios vary from 0.027 with the Grundy silt loam from Illinois to 0.339 with the Norfolk sandy loam from Alabama. In other words, the former soil has 2.7 per cent as much potassium as calcium in the soil solution, whereas the latter at the same pH value has 33.9 per cent as much potassium as calcium. It will also be observed that there is a fair correlation between the ability of plants to grow on the soils and the potassium-calcium ratio. Two soils prove exceptions to a close correlation, soils 679 and 683. In both these soils a fairly narrow ratio is not accompanied by very poor plant growth. It is possible, however, that magnesium and sodium should also be considered, and that the ratios of sodium and potassium to calcium and magnesium would show a better correlation with plant growth than does the potassium-calcium ratio.

The percentage base saturation of soils as related to the growth of plants on acid soils

The recent excellent studies in Europe and in this country on the base exchange phenomena in soils have led to a better understanding of the nature of soil acidity. It is now generally recognized that soils are acid because of the presence of exchangeable hydrogen. Determinations of the total amount of exchangeable hydrogen of soils, however, cannot serve as a measure of plant response to liming, because most plants can make optimum growth on soils that are not completely neutralized. Since, however, the exchangeable bases are believed to be more readily available to plants than are those in the non-exchangeable form and since soil acidity is believed to be related to calcium

deficiency, it has been proposed that the amount of exchangeable calcium is a criterion for the ability of plants to grow on acid soils. It seems to the writer, however, that the amount of exchangeable calcium, considered by itself, is of much less significance than is its consideration in relation to the exchangeable hydrogen present. If that is true, then one would expect a correlation between the percentage base saturation of soils and plant growth on acid soils.

The percentage base saturation of the soils used in this study was determined; and the values obtained were studied in relation to the growth of plants on these soils. The data are presented in the next to the last column of table 5. As is shown in a paper by Scarseth and the writer (35), the soils vary considerably in their percentage base saturation at similar pH values. This is well shown in table 6 where values are given for the soils at pH 4.80.

There seems to be very little correlation between the depression in yield of corn and the percentage base saturation values. Although the two soils which have the highest percentage base saturation are also those which give the least depression in yields, some soils of low percentage base saturation give very little depression in yields. It must be remembered, however, that with only two of the soils was there much injury to corn from soil acidity. For this reason, the data for this crop are not very conclusive.

Tables 5 and 6 show that with sorghum there is a better, although by no means perfect, correlation between the percentage base saturation of soils and plant growth at low pH values. The most striking example of the correlation is probably for soils 670 and 673. These two soils produce as good growth of sorghum at pH 4.75 and 4.60 respectively as at any higher pH value. For soil 673, at least, these results cannot be explained on the basis of the concentration of aluminum in solution, for this soil contains as much soluble aluminum at pH 4.60 as do other soils which showed decided injury. It will be noted, however, that at these low pH values these two soils are still very highly saturated with bases. All of the other soils which show injury to the growth of sorghum at similar pH values have a much lower degree of saturation. These results, no doubt, explain the fact that soil 670, the Grundy silt loam from the Aledo Experiment Field in Illinois, responds very little to liming in the field although its pH value is between 5.00 and 5.20. Another example of the relation between the percentage base saturation and plant growth is with soils 675 and 683. The difference in yields of these two soils at high degrees of acidity could not be explained on the basis of hydrogen-ion concentration nor of aluminum toxicity. On the basis of percentage base saturation, it will be noted that that the differences in the extent of the injury might be explained.

The yields of barley also show that there is a better correlation between the depression in yields and the percentage base saturation of the soils than between the depression in yields and the concentration of aluminum in the displaced soil solutions. Again there are some exceptions to this correlation. Soils 680 and 678, for example, produced less depression in yields of both the sorghum and the barley crops than would be expected from their percentage base satura-

tion. The lack of a better correlation, however, is not surprising. In the first place, small differences in yields or in the percentage base saturation values cannot be considered significant. Moreover, the injurious action of soil acidity on plant growth is undoubtedly affected by more than one factor.

The correlation that exists between the plant injury to corn on the different soils and the concentration of aluminum in the soil solution, and the indications of some correlation between the aluminum concentration and plant injury to sorghum and barley would suggest that this is probably not incidental, but that soluble aluminum is at least a secondary factor in explaining plant injury on acid soils. Evidence for this is found in these data in the correlation between plant growth and a combination of aluminum concentration and percentage base saturation as compared with the correlation between plant growth and either of these values alone. Where growth is better than would be expected from the percentage base saturation of the soil, the concentration of aluminum in the displaced solution is relatively low; whereas when the growth is poorer than would be expected from the percentage base saturation, the concentration of aluminum in the soil solution is relatively high. For example, soil 675 gave a lower yield than would be expected from the percentage base saturation value as compared with soil 674, but it will be noted that it contains much more aluminum in its displaced solution than does soil 674. Soil 680, on the other hand, gave better yields at pH 4.80 than would be expected from its percentage base saturation when compared with soils 671 and 676, but it contains less aluminum in solution than do the other two soils. It seems, therefore, that the percentage base saturation at which plants are injured on acid soils is affected somewhat by the concentration of aluminum in the displaced solution. Where the concentration of aluminum in the displaced solution is high, plant injury from soil acidity will occur at higher degrees of saturation than when the concentration of aluminum is low. Because of the fact, also, that old weathered soils usually have a lower degree of saturation at given pH values than younger soils, it will be noted that in general the soils of the Piedmont Plateau and Coastal Plain provinces showed a greater injury from acidity at relatively low concentrations of aluminum than did the other soils studied.

GENERAL DISCUSSION

The data presented in this paper show that the percentage base saturation of soils is a better criterion for the growth of sorghum and barley on acid soils than is hydrogen-ion concentration or soluble aluminum. It is also believed from a consideration of these and other data that the percentage base saturation of a soil is of more significance than is the amount of exchangeable hydrogen or the amount of exchangeable calcium present. In this connection the following investigations are of interest. Robinson and Williams (37) found that some soils which gave distinct indications of acidity but did not respond to liming contained 0.2 per cent or more of exchangeable calcium, whereas others that responded to liming contained smaller amounts of exchangeable calcium.

They believe, therefore, that the available calcium is the important factor influencing plant response to liming on the organic soils with which they worked. Rost (38), however, found little correlation between the exchangeable calcium removed by electrodialysis and crop response to lime.

Duley (12) and Fleetwood (13) determined the amount of soil calcium soluble in 0.04*N* carbonated water. This amount of calcium, as was later shown by Robinson and Williams, is smaller than the amount of exchangeable calcium, but it bears a relationship to the exchangeable calcium. Duley and Fleetwood have shown that there is a relationship between the calcium soluble in carbonated water and the response of plants grown on these soils to liming. For all the soils studied, Fleetwood found that those having less than 650 pounds of calcium to the acre gave good responses, whereas those having more than 700 pounds gave poor responses to liming.

It hardly seems possible, however, that if soils varying considerably in texture or total exchange capacity are considered, a correlation would be obtained between the exchangeable or carbonate-water soluble calcium and plant response to liming. For example, many sandy Coastal Plain soils have a total exchange capacity of less than 3 mgm. equivalents per 100 gm. of soil. These soils at pH 6.0 are often between 50 and 60 per cent saturated with bases. This means that the exchangeable calcium is less than 2 mgm. equiv. And yet, most crops on these soils do not respond to liming. Even when they are fully saturated with bases, they contain less than half as much exchangeable calcium as some soils of a heavier texture or of greater total exchange capacity which are very acid and in need of lime. It seems evident, therefore, that the amount of calcium soluble in carbonated water or the amount of exchangeable calcium cannot serve as a safe indication of whether a soil needs to be limed for the successful growth of crops, unless these values are considered in relation to texture or, better still, to the total exchange capacity of soils. It is very probable that the reason Robinson and Williams (37) obtained correlation between exchangeable calcium and plant response to liming, whereas Rost (38) did not, is that Robinson and Williams worked with soils of much the same type, whereas Rost worked with soils of widely different textures and total exchange capacity. Some of the data obtained by Rost can probably be explained on the basis of percentage base saturation. To quote from his statements: "Soil 37 and 22 show nearly the same percentage increase in yield from liming, but the larger amount of lime is removed from the finer textured soil." It is evident that this larger amount of lime removed from the finer textured soil may represent the same or even a lower degree of saturation than does the smaller amount extracted from the coarser soil.

The percentage base saturation viewpoint of the relation between plant growth and soil acidity helps to explain some other facts observed by various investigators. Thus it has been observed by Conner (10), among others, that acid soils which have a high colloidal content are capable of producing good crops even though several tons of lime to the acre are required to bring

them to neutrality. Soils high in colloids have a high total exchange capacity. Therefore, the presence, for example, of 4 mgm. equiv. of hydrogen, which would theoretically require 2 tons of lime an acre for neutralization, leaves a soil that has a total exchange capacity of 20 mgm. equiv. 75 per cent saturated; whereas the presence of that same amount of acid in a soil containing less colloid, such as one having a total exchange capacity of 5 mgm. equiv. would leave that soil only 20 per cent saturated. Moreover, it is interesting to note in this study that there is in general a positive correlation between the total exchange capacity of the soils and their crop producing power. This may partly explain the statement sometimes made that fertile soils are able to withstand greater degrees of acidity than poorer soils. The failure to get response from liming on soils that are quite acid, therefore, may be due to the fact that such soils, although acid, have a high degree of saturation.

The results obtained by Alway and Nygard (2) and by Sewell and Perkins (40) can probably also be explained on the basis of percentage base saturation.

One of the first explanations that was proposed by soil investigators to account for the injurious action of soil acidity on plant growth was that plants in acid soils cannot get sufficient available calcium. The fact, however, that calcium added to acid soils in the form of calcium sulfate did not overcome the injurious action of these soils on plant growth was difficult to explain, for if soluble calcium were present it seemed as if it should be available. Truog (44) in his earlier comprehensive study of soil acidity in its relation to plant growth suggested that the reason calcium sulfate did not prove beneficial on acid soils was that plants needed calcium not so much as a plant nutrient but to neutralize the acids formed within the plant. He concluded, therefore, that the calcium, to be of value, must be present in soils in the carbonate or bicarbonate form. He further concluded that the amounts of calcium which can exist in acid soils in the bicarbonate form are to a large extent determined by the amount and strength of the soil acids present. At the time of his study little was known regarding the base exchange reaction of soils. It is significant, however, that in a large measure his theory is compatible with the percentage base saturation conception. In his more recent studies Truog (45) has emphasized solid phase feeding by which he believes that the calcium in the base exchange compounds may serve the same purpose as that in the carbonate or bicarbonate form.

It may also be that the amount of calcium which can be absorbed by the plant is dependent on other factors such as the relative concentration of calcium to other ions in solution. According to this viewpoint, the fact that calcium sulfate is not beneficial when added to an acid soil could be explained on the basis that it not only adds more calcium to the soil solution but that through the base exchange reaction it liberates considerable hydrogen from the soil complex into the soil solution.

Another factor which probably affects plant growth on acid soils and one that is closely correlated with percentage base saturation is the proportion of

the various exchangeable bases in different soils at similar degrees of base saturation. Although it has been shown by Kelly and Brown (16) and others that the predominant exchangeable base in soils from humid climates is calcium, no one, as far as the writer knows, has studied the relative proportion of the various bases present as soils become progressively more desaturated. It is possible, for example, that two soils may have about the same proportion of the various exchangeable bases at pH 6.5, but that as they become progressively more desaturated the relative proportion of calcium to the other bases maybe quite different. Calcium or magnesium may become a limiting factor much sooner in one of these soils than in the other even though both soils have the same degree of saturation. Moreover, one of the bases may become limiting not only because its total concentration is in itself low, but because it is low in proportion to the other bases present. That such may be the case is indicated by the data on the potassium and calcium content of the soil solutions. Although there was found to be no relationship between plant injury and the amount of calcium in solution, some correlation was obtained between plant injury and the ratio of potassium to calcium in the soil solution. It is possible that the correlation would have been even better if magnesium and sodium had also been determined and if these ratios had been determined for the bases in the exchangeable form.

In this connection the investigations of Cooper and Wilson (11) are of interest. They have proposed the theory that the absorption of ions by plants is influenced by their oxidation-reduction potential. Thus, potassium, which has a high oxidation-reduction potential, would be absorbed much more readily than calcium if present in equivalent concentration. A relatively large amount of potassium compared with calcium, as was found in this study for the soils showing greatest plant injury, would mean that potassium is probably absorbed to the partial exclusion of calcium, even though the latter is present in solution in quite high concentrations.

The recent results obtained by Brown (5) in culture solutions are also of interest. He found that if the molecular ratio of calcium nitrate to potassium di-hydrogen phosphate is decreased from 4:1 to 3:2, a marked reduction in growth is obtained. With the reduction in growth the calcium-potassium ratio in the plant changed from about 1:1 to about 1:4. He also found that increasing the concentration of potassium in the solutions greatly depressed the absorption of magnesium by the plant, even though this element was present in both solutions in the same concentration. The injury to the plants from a relatively high absorption of potassium as compared with calcium and magnesium may be because, as found by True (43), it replaces these elements in the pectates of the middle lamella, causing the cell wall to become more permeable.

These considerations give added reason for believing that the percentage base saturation of soils, and probably the proportion of the various bases present in the exchange complex and in the soil solution, are primary factors which directly influence plant growth on acid soils.

SUMMARY

This investigation was conducted in the greenhouse with 13 soils formed under different climatic conditions and of different degrees of weathering and obtained from several states. Each was brought to five different degrees of acidity by appropriate lime and acid treatments. Crops of corn, sorghum, and barley were grown on the soils. The results of the investigation can be briefly summarized as follows:

The "critical hydrogen-ion concentration" for the growth of crops varied considerably with different soils; therefore, the hydrogen-ion concentration of soils cannot be considered the direct cause of poor plant growth nor the main factor governing plant distribution or response to liming.

The displaced soil solutions of the soils were analyzed for aluminum, calcium, potassium, manganese, and phosphorus.

Aluminum was found present in very small concentrations even in the most acid soils.

The concentration of aluminum in the displaced solution of the different soils at similar pH values varied considerably.

There was some evidence of correlation between the concentration of aluminum in the displaced soil solution and the growth of corn on acid soils but less correlation between the concentration of aluminum and the yield of sorghum and barley. The data are taken to indicate that soluble aluminum is not the primary factor in influencing the growth of acid-sensitive crops on acid soils. Some evidence is given for the belief that it is a secondary factor.

No relation was found between the amount of phosphate in the soil solution and the concentration of aluminum in solution at which plant injury developed.

The concentration of calcium in the soil solution bore no relationship to plant injury on these soils.

A fair correlation was found to exist between the ratio of potassium to calcium in the soil solution and plant injury. As the soils became more acid, the ratio of potassium to calcium decreased. This ratio varied considerably for the different soils at similar pH values.

No relation was found between the concentration of manganese in solution and plant injury from soil acidity.

The determinations of the percentage base saturation of the soils gave interesting results.

Soils varied considerably in their percentage base saturation at similar pH values.

A rather good correlation was found to exist between the percentage base saturation of acid soils and plant injury. Where large amounts of aluminum were present in solution, plant injury seemed to occur at higher degrees of saturation than when aluminum was absent, or present in low concentrations.

The percentage base saturation of soils is believed to be one of the most important factors in determining the growth of plants on acid soils. It is also suggested that differences in the relative proportion of the various bases present in the exchange complex and in the soil solution may be contributing factors to the poor plant growth obtained on acid soils.

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CHANGES IN THE AVAILABILITY OF PHOSPHORUS IN IRRIGATED RICE SOILS[1]

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Phosphatic fertilization of rice, as a rule, has not been profitable in Arkansas on soils which are known to be deficient in available phosphorus. The increased yields, if any, are not large enough to repay the cost of the fertilizer. Why should rice fail to respond to phosphatic fertilizers on soils which produced marked increases with applications of phosphorus-carrying fertilizers to other crops, such as soybeans?

A hypothesis commonly offered by rice growers to explain the condition mentioned, is that phosphatic fertilizers stimulate weed growth to such an extent that the weeds can not be controlled by irrigation. As a consequence, a considerable amount of the plant nutrients are used up by the weeds and no increased yields of rice are obtained. This hypothesis, although logical, is based largely on field observations and does not consider the abnormal conditions which develop in the soil after the rice is irrigated. Neither does it consider the fact that phosphatic fertilizers on rice have been reported as profitable in some instances in other countries (6) and (12). A survey of the conditions under which rice is grown suggests that a number of factors other than weed growth may be active in affecting the availability of phosphorus in rice soils.

Rice in Arkansas is grown in a region underlain by limestone rocks. As a result the water from wells used for irrigation may contain considerable amounts of calcium which could cause phosphorus in the soils to revert to insoluble forms. These are unavailable for rice, which, as has been previously shown (1), is a very poor feeder on insoluble phosphates. The development of anaerobic conditions after irrigation might cause some of the available phosphorus to be changed to forms which are unavailable for rice.

The investigation reported herein was started in an effort to study some of the factors which may affect the availability of phosphorus under the conditions of rice production and to determine the manner in which those factors act.

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PLAN OF EXPERIMENT

The work was planned to determine by chemical analyses the reaction, the calcium content, and the iron and aluminum content of water from wells used for irrigation purposes; to determine by chemical analyses the reaction of surface and subsoils devoted to rice production; and to study under controlled conditions the effect of irrigation upon the availability of phosphorus in rice soils which had received different fertilizer treatments.

Calcium, iron, and aluminum content of irrigation waters

A 2½-liter sample of water was taken as it came from the pump from each of several different wells in the rice area when they were pumping water for the irrigation of rice fields. The samples were brought to the laboratory and their calcium, iron, and aluminum content and reaction were determined. Iron and aluminum were not determined separately, inasmuch as their effect on soluble phosphorus compounds would be similar. In addition, the reaction of water from several wells from which no samples were taken for analyses was determined colorimetrically. The results of the analyses are given in table 1.

The reaction of all samples was either neutral or alkaline. The suggestion, given by the alkaline reaction of the water, that they might contain considerable amounts of basic material in solution is proved by the results of the chemical analyses. The amounts of calcium, although variable, are fairly large. The results are very consistent and agree with results of analyses made by the United States Geological Survey (13). Their results showed the calcium content to vary from 22 to 112 p.p.m., whereas the iron content was from 0.55 to 6.56 p.p.m. The calcium content varied from 20.3 p.p.m. to 74.8 p.p.m., although most of the results were near the latter figure. The amounts of iron and aluminum are likewise variable, but are present in appreciable amounts, varying from 6.1 to 12.8 p.p.m.

The full significance of the presence of calcium, iron, and aluminum in the irrigation water is seen when the amounts added annually in the irrigation water are calculated. According to the preliminary results of investigations by Carter and Clayton (3), the net duty of water in the rice area is 22.3 inches. Because of an impervious subsoil, vary little of this water is lost by seepage. It is largely removed by transpiration of the plants and evaporation from the surface of the water. As a result, practically all of the basic material applied in the irrigation water is deposited in the soil. The amounts applied annually are given in table 2.

In all but two of the samples, more than 300 pounds of calcium, free to combine with either insoluble soil acids or the more soluble forms, such as phosphoric and sulfuric acids, are added annually. In addition, from 30 to 60 pounds of soluble iron and aluminum are added. Thus rice soils, cropped successively to rice for a period of years because of the expense involved in

establishing irrigation systems, receive an enormous amount of soluble basic materials in a relatively short time.

These materials added in the irrigation water could greatly decrease the supply of available phosphorus. A residue of 194 pounds of calcium would

TABLE 1

Analyses of well waters used for irrigation of rice fields

SAMPLE NUMBER	pH	Ca IN WATER	Fe_2O_3 AND Al_2O_3 IN WATER
		p.p.m.	*p.p.m.*
1	7.5		
2	7.5		
3	7.0		
4	7.5		
5	7.5		
6	7.5		
7		24.5	
8	7.2	66.8	11.6
9	7.3	20.3	12.8
10	7.3	74.8	12.4
11	7.4	66.2	12.0
12	7.2	70.0	8.5
13	7.4	66.4	6.1
14	7.0	51.4	6.2
15	7.2	66.8	8.9
16	7.3	72.3	6.4
17	7.4	70.9	11.6

TABLE 2

Approximate amounts of calcium and iron and aluminum added to the soil annually by irrigation water

SAMPLE NUMBER	Ca ADDED PER ACRE	Fe_2O_3 AND Al_2O_3 ADDED PER ACRE
	pounds	*pounds*
8	325	56
9	98	62
10	363	60
11	320	58
12	339	41
13	321	29
14	249	30
15	323	43
16	350	31
17	343	53

have the ability to revert 150 pounds of available phosphorus in the soil, if it existed as the monocalcium salt. This is considerably more than most soils contain and is more than the amount contained in one ton of superphosphate. This does not necessarily mean that such a reaction takes place, but merely

illustrates the enormous fixing power of the material added to the soil each year. On the other hand, this basic material might be used to neutralize soil acids. However, even if the added basic materials were utilized largely in neutralizing both soluble and insoluble soil acids they would still increase the amount of replaceable base in the soil. The ultimate effect on the soluble phosphorus compounds would be the same. This is in excellent agreement with the results of Metzger (7), who has shown that continued irrigation has greatly increased the amount of replaceable calcium in the surface soil.

Effect of irrigation on soil reaction

The annual addition of such large amounts of basic materials to soils should result in the soils becoming more alkaline. Although determinations of the

TABLE 3

Effect of irrigation on the reaction of rice soils

SAMPLE NUMBER	SURFACE SOIL pH	SUBSOIL pH	SAMPLE NUMBER	SURFACE SOIL pH	SUBSOIL pH
1	5.6	5.2	17	7.0	6.8
2	5.9	*	18	5.2	5.3
3	6.2	*	19	6.4	6.6
4	6.7	5.9	20	6.8	5.9
5	7.4	*	21	6.7	5.6
6	6.1	*	22	6.8	6.5
7	6.9	*	23	6.6	5.8
8	6.3	6.0	24	7.1	6.3
9	7.4	6.6	25	6.7	6.0
10	7.0	6.3	26	6.4	5.8
11	5.5	5.6	27	6.6	6.1
12	6.1	5.5	28	7.0	6.5
13	7.1	6.2	29	6.6	5.4
14	6.1	5.8	30	6.7	5.7
15	7.0	5.3	31	6.5	4.9
16	6.4	6.0			

* Subsoil not sampled.

reaction of soils some years after irrigation without a definite knowledge of the initial reaction of the soil might give misleading results, the conditions in the rice area of Arkansas are such that the general trend of such changes may be obtained. The soil is a rather heavy silt loam underlain by an impervious subsoil. The subsoil is so impervious that very little of the irrigation water is lost by percolation. As a result, most of the materials carried by the irrigation water are deposited in the surface layer. The surface soil, therefore, should become more alkaline than the subsoil. The reactions of a number of surface and subsoils taken from farms within a radius of 20 miles in the rice district are given in table 3.

The surface soil was taken from the surface 6 inches and the subsoil from the

12 to 18-inch layer. Sample 11 has been in rice only one year. Samples 18 and 19, from virgin soil, illustrate the conditions generally found in virgin soils of this type, viz., the reaction of the surface and subsoil are almost the same. The results show very clearly the tendency of irrigation to make the surface more alkaline. In some cases, 4, 9, 10, 13, 15, 17, 23, and 28, the soil acids have been completely neutralized whereas in many of the others it has been almost all neutralized. While neutralization was taking place, the abundance of basic materials was undoubtedly reverting some of the phosphorus in the soil and making it unavailable for the growth of rice.

Water-soluble phosphorus in rice soils

To make normal growth, all plants need a certain amount of phosphorus. If the amount coming from the soil is insufficient for normal needs, the amount of growth is reduced. With the addition of so much basic material in the irrigation water, it is important that the amount of phosphorus in the soil solution be known. A knowledge of the ability of the soil to maintain that concentration is of equal importance.

Analyses for phosphorus by the ceruleo-molybdate method of Deniges (5), as modified by Parker and Fudge (9), in the displaced soil solution showed it to contain 0.75 p.p.m. of total PO_4, of which 0.3 p.p.m. was in the inorganic form, and, according to Pierre and Parker (11), is available to plants. From our present knowledge of phosphorus requirements of plants (8) 0.3 p.p.m. inorganic phosphorus would enable plants to make normal growth provided that concentration was maintained.

The soil solution had a pH of 7.2, indicating the presence of considerable basic material. Qualitative analyses showed the presence of considerable calcium.

In order to test the ability of the soil to maintain its original supply of available phosphorus, an experiment was performed to detemine the rate at which renewal of phosphorus in the soil solution would take place. The amount of inorganic phosphorus coming into solution during various intervals was determined by the collodion bag method (10). Thirty grams of soil was placed in the collodion bags and 50 cc. of water added. One hundred cubic centimeters of distilled water was added to the outside. At each sampling, the 100-cc. portion on the outside was removed for analysis and another 100 cc. of distilled water added to the flask. The inorganic phosphorus was determined, as given in the foregoing and the pH was determined colorimetrically. Because of the possibility that the abundant bacterial growth which developed inside the collodion bags might have affected the availability of the phosphorus, a second set was run in which an effort was made to control bacterial growth by the addition of a few drops of toluene. The results of both experiments are given in table 4.

The results show that while the concentration of inorganic phosphorus in the soil extract is low, 0.13 p.p.m., the soil is able to maintain the amount in

solution at a rather uniform level. On the other hand, factors such as the rapid development of microorganisms may cause a very rapid depletion of the inorganic phosphorus in solution. This occurred after 8 days, where no toluene was added, and at the end of 10 days when toluene was added at the beginning of the experiment. Constant removing and renewal of solution had probably depleted the supply of toluene added at the beginning of the experiment. The microorganisms developed so rapidly that in a few days they caused the liquid outside of the collodion bags to become cloudy.

There is a suggestion in the results of the second experiment of the repression caused by the basic materials added in the irrigation waters. There is a gradual decrease in the water-soluble phosphorus for a time, followed by an increase as the extraction continued. Accompanying these changes, there is a gradual increase in pH from 6.5 to 7.3, presumably because of basic material coming into solution. This is in agreement with Burd and Martin (2), who state that as the cations are removed from a soil more phosphorus will go into solution.

TABLE 4

Inorganic PO_4 in successive diffusates from 1:5 ratio of rice soil and water

TIME	INORGANIC PO_4 IN DIFFUSATE	PO_4 DISSOLVED IN INTERVALS BETWEEN SAMPLING	pH
hours	*p.p.m.*	*p.p.m.*	
	Without toluene		
24	0.13	0.13	6.7
48	0.13	0.09	...
84	0.08	0.04	...
108	0.08	0.05	6.0
132	0.08	0.05	...
180	Trace	—	6.4
	With toluene		
24	0.08	0.08	6.5
48	0.07	0.05	6.7
72	0.06	0.04	6.8
120	0.09	0.07	7.2
144	0.08	0.05	7.2
168	0.10	0.08	7.3
240	Trace	—	7.2

The relatively small amount of inorganic phosphorus found in the soil solution readily accounts for the marked response of other crops than rice where applications of soluble phosphatic fertilizers are made to these soils.

Changes in availability of phosphorus produced by irrigation

In addition to the effects given in the preceding experiments, there is also the possibility that the anaerobic conditions caused by the irrigation of the

TABLE 5

Amounts of PO_4 in 1:5 water extracts of soils with the fertilizer and irrigation treatments indicated

TREATMENT		PO_4 IN EXTRACT								
Fertilizer	Irrigation	May 6			June 8			July 22		
		Inorganic	Organic	Total	Inorganic	Organic	Total	Inorganic	Organic	Total
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
None	None	0.06	0.30	0.36	Trace	0.14	0.14	None	0.14	0.14
None	Flooded with distilled water	Trace	0.46	0.46	Trace	0.21	0.21	None	0.55	0.55
250 pounds $CaH_4(PO_4)_2$	Flooded with distilled water	0.09	0.43	0.52	0.11	0.17	0.28	Trace	0.64	0.64
200 pounds $(NH_4)_2SO_4$	Flooded with distilled water	Trace	0.38	0.38	Trace	0.32	0.32	None	0.44	0.44
250 pounds $CaH_4(PO_4)_2$	Flooded with calcium hydroxide* solution	0.07	0.47	0.54	Trace	0.35	0.35	Trace	0.74	0.74
None	Flooded with calcium hydroxide solution	Trace	0.61	0.61	Trace	0.28	0.28	None	0.46	0.46

* Calcium equivalent to 300 pounds an acre was added in first four applications and distilled water was used for later irrigations.

rice fields may produce a change in the kind or amount of water-soluble phosphorus in the soil. A series of jars containing equal amounts of soil, but receiving different treatments, were started in the greenhouse to study the effect of irrigation on the amount and forms of water-soluble phosphorus in the soil. Clear solutions of 1:5 water extracts were obtained by the collodion bag method. They were analyzed colorimetrically for organic, inorganic, and total water-soluble PO_4. The fertilizers were added and the soils flooded April 5, 1929. The treatments and results of the analyses of different samplings are given in table 5.

Flooding has apparently caused relatively rapid fixation of the soluble inorganic phosphorus in the soil solution. Only one month of flooding has caused the disappearance of nearly all of the available phosphorus. This has taken place even where mono-calcium phosphate was added at the rate of 250 pounds an acre. This was probably due to reversion of the phosphate.

The effect of the increase of basic material in solution is also seen in the change in pH from the first and second sampling. At the first sampling, the unflooded check had a pH of 6.1 whereas the others had a pH of 6.3. On June 8, the acidity of the unflooded check had increased to pH 5.72, whereas all the others decreased to between pH 6.92 and pH 7.18. This change, except in the two treatments receiving calcium hydroxide in the irrigation water, could be attributed only to the basic material coming into the solution.

It is also shown that part of the phosphorus may have been converted to an organic form. As a result of the suggestion given from the results of the collodion bag experiments an effort was made to observe the changes, if any, in biological activities by means of the Conn direct staining method (4). Although differences in type of organisms were undoubtedly present, they were not outstanding enough to be observed by the writer. The amount of organic phosphorus in the extract for the July 22 analysis shows a decided increase in all of the flooded treatments, further supporting the idea that inorganic phosphorus may be transformed to organic phosphorus. This also may be a factor concerned in the phosphatic fertilization of rice.

The completeness of reversion is shown by the following experiment. After the July 22 sampling, the moisture content was reduced by normal evaporation to 10 to 18 per cent and maintained in that range for six months. At the end of the time, no more than a trace of water-soluble inorganic phosphorus was found in any jar.

DISCUSSION OF RESULTS

The maintenance of an adequate supply of plant-food constituents for good yields of crops is the primary object of fertilizer practices. Any factors which may affect the supply of available plant-food will naturally affect the practices operating in the fertilization of crops.

Admittedly the application of phosphatic fertilizers to soils deficient in phosphorus will stimulate weed growth just as it does crop growth. If the

weed growth is not controlled, it is self evident that there will be competition between weeds and rice, which is a poor forager for available phosphorus, with the stronger feeder getting the major portion of the available phosphorus.

On the other hand, it seems quite evident that if other crops can compete successfully on the same soil with weeds and make profitable increased yields with applications of phosphatic fertilizer, then poor returns from the use of phosphatic fertilizers on rice must be linked in some way with this particular crop.

The application of large amounts of basic materials annually in the irrigation water used on rice has resulted in reducing the hydrogen-ion concentration of soils very much. In some instances, the change has been from strongly acid to a decidedly alkaline reaction. The result has been that phosphorus has reverted to slightly soluble forms and is only slowly available to rice, which is a weak feeder on insoluble phosphates.

At the same time, irrigation of the soils causes an increase in replaceable bases in the soil, thus causing reversion of the more soluble phosphate. As a result, a few weeks after irrigation, the amount of inorganic phosphorus in the soil may be practically nil. Reversion takes place even when soluble phosphatic fertilizers are added to the soil.

The anaerobic conditions produced by the irrigation may cause a change in the type of inorganic forms of water-soluble phosphorus, which are available to plants, to organic forms which are not available to plants.

The action of most factors affecting soil conditions in the production of rice is to decrease the solubility of phosphatic fertilizers. From the results of these experiments, it does not seem advisable to recommend the use of phosphatic fertilizers where the land is irrigated with calcareous water, until some means is found to keep the phosphorus in solution.

SUMMARY

Studies were made under field conditions to determine the effect of irrigation on the pH of rice soils. The calcium content, iron and aluminum content, and pH of well water used for irrigation puposes were found by analysis. The effect of irrigation on the availability of phosphorus in soils receiving different fertilizer treatments was studied. The results of the investigation may be summarized as follows:

Water used for the irrigation of rice in Arkansas contains large amounts of calcium, largely in the bicarbonate form.

From 6 to 12 p.p.m. of soluble iron and aluminum were found in the samples analyzed.

The amounts of soluble calcium and iron and aluminum added annually in the irrigation water could cause reversion of large amounts of soluble phosphorus.

Continued irrigation with calcareous well water has made the surface soil in the rice area decidedly more alkaline than the subsoil.

Irrigation caused a descrease in the water-soluble inorganic phosphate. This was partially due to reversion of the phosphates.

The increase in the organic water-soluble phosphorus three months after irrigation may be due to activities of bacteria under anaerobic conditions.

Phosphatic fertilizers, according to the results of these experiments, should not be recommended when calcareous water is used for irrigation.

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THE INFLUENCE OF COMBINED NITROGEN ON GROWTH AND NITROGEN FIXATION BY AZOTOBACTER

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It is commonly assumed that available combined nitrogen in the substrate will inhibit nitrogen fixation by Azotobacter. Mockeridge (23) says: "It is a well known fact that when supplied with soluble nitrogen, Azotobacter does not fix atmospheric nitrogen until the available nitrogen has been consumed."

This assumption is apparently accepted by many investigators in the field of Azotobacter physiology. The evidence in the literature, however, hardly seems adequate to justify the unqualified acceptance of the idea that the presence of such nitrogenous materials in the soil or other substrate will necessarily depress nitrogen fixation by Azotobacter. In fact, some of the investigations reported point in the opposite direction.

Krainski (18) observed that nitrogen fixation is more active in rich or well-fertilized soils than in poor soils. Löhnis and Green (20) employed a synthetic medium to which they added manure, straw, and peat. They inoculated this medium with Azotobacter and observed that nitrogen fixation was increased. Greaves and Carter (9) found that the treatment of soil with stable manure under field conditions had a beneficial effect upon the nitrogen-fixing power of the soil. Large quantities of manure did not prove as beneficial as smaller quantities.

Richards (26) investigated the effect of garden soil when added to fresh horse or cow manure. He found that the nitrogen content of the mix increased during incubation, and isolated Azotobacter and *B. lactis-aerogenes* from the cultures. Greaves and Nelson (10) state that " . . . . commercial fertilizers may discourage the beneficial bacteria, including nitrogen-fixing organisms, to such an extent that they cease to gather nitrogen from the air, whereas the use of farmyard manure may encourage them." They further suggest that manure carries phosphorus, potassium, and other food to the soil. These materials may be as much responsible as the nitrogen for any stimulating influence on nitrogen fixation.

Beijerinck and Van Delden (3) showed that Azotobacter has the power to attack nitrates, converting them to ammonia. Lipman (19), in a study of *Azotobacter vinelandii*, tried the effect of potassium nitrate, peptone and ammonium chloride. He found that available nitrogen reduced the fixation of atmospheric nitrogen. Stoklasa (30), Stranak (31), and Heinze (12) showed that small quantities of nitrates stimulated nitrogen fixation by Azotobacter.

Hills (13) tried the effects of sodium, potassium, and calcium nitrates on soil cultures of Azotobacter. He employed 150 gm. dry weight of soil as the medium and added the nitrates in quantities of from 10 to 300 mgm. to each culture, with 1 per cent mannitol as a source of energy. The numbers of Azotobacter increased in cultures containing up to 100 mgm. of sodium, potassium, and ammonium nitrates, and 50 mgm. of calcium nitrate. Maximum nitrogen fixation was observed with 100 mgm. of sodium and potassium nitrate in the case of one Azotobacter strain, and with 150 mgm. of each with another strain of the organism. In every instance the use of calcium nitrate caused a marked decrease in the amount of nitrogen fixed.

Hills found, further, that sterilized soil containing sodium nitrate in quantities of 50 and

150 mgm. to 100 gm. of soil showed at least three times as much nitrogen fixation as the controls. There was not much difference between the two concentrations. In un-sterilized soil 50 mgm. gave a marked increase in nitrogen fixed, whereas with 150 mgm. almost twice as much was formed.

Tests in sterilized soil showed a marked increase in nitrogen fixation with 50 mgm. of calcium nitrate in 100 gm. of dry soil, but not quite as much as with 200 mgm., the proportion being 7.8 to 9.8 mgm. of nitrogen fixed. In un-sterilized soil the results were similar when 50 mgm. of the nitrate were added, whereas 200 mgm. showed a fixation of 12.8 mgm. of nitrogen in 100 gm. of soil. The nitrogen was found as organic nitrogen. Any loss resulting from the attack on the nitrate is made up by the fixation of free atmospheric nitrogen.

The percentage increase in the numbers of viable Azotobacter in these experiments was about 10 times as great as the maximum nitrogen increase. Hence, the results show that the physiological efficiency of the Azotobacter, in so far as fixation of atmospheric nitrogen is concerned, is actually decreased to a marked degree.

Bonazzi (4) found that the addition of calcium nitrate not only resulted in the inhibition of nitrogen fixation by the organism, but that there was some loss of the nitrogen from the nitrate incorporated in the medium.

Zoond (37) observed that nitrogen fixation was depressed when potassium nitrate, tyrosine and Bacto-peptone respectively were added to a medium in quantity sufficient to give a nitrogen concentration of more than 0.003 per cent. Maximum fixation occurred in a concentration of 3 mgm. of nitrogen in 100 cc. of solution. The drop in nitrogen fixation was rapid as increasing quantities of the substance were added. The investigator suggests that the apparent stimulus resulting from small quantities of nitrogenous materials may be due to the law of concentration of ions, as observed by Winslow and Falk (36) in the use of increasing quantities of sodium chloride and calcium chloride on the rate of dying of *B. coli*. Zoond adds his suggestion to that of Bonazzi, that nitrogen fixation is a reserve power resorted to by Azotobacter only in case of nitrogen starvation.

McBeth (21) investigated the effect of 0.1 per cent ammonium sulfate on the nitrogen-fixing activities of *Azotobacter chroococcum* and *A. beijerinckii*. He concluded that the presence of ammonium sulfate stimulates nitrogen fixation by the organisms. He made no mention of the amount of growth. He conducted qualitative, but not quantitative, tests for ammonia.

Reed and Williams (25) tried the effects of various organic nitrogen compounds, including caffeine, trimethylamine, asparagine, creatinine, xanthine, hypoxanthine, urea, formamide, glycocoll, allantoine, guanidine carbonate, and skatole. They found that nitrogen fixation was only slightly affected by most of the compounds investigated. Guanidine and skatole were toxic, whereas caffeine appeared to stimulate the growth of Azotobacter. Urea, glycocoll, formamide, and allantoine were especially active in depressing nitrogen fixation, because the authors suggest, of their satisfying the nitrogen demands of Azotobacter, rather than because of any toxic effect. The simpler substances appeared to exercise the most depressing effect. This might be expected because they probably are the more easily utilized.

It seems logical that, as proteins are decomposed in the soil, the degradation products, including amino acids, should be present in definite but varying quantities. Amino-acid nitrogen was first demonstrated in soil by Warrington (34) and later by Sestini (29). Dojerinko (7) made quantitative estimates of the amounts of monamino and amido nitrogen in humic acid extracted from Russian soil. Jodidi (14) first demonstrated the presence of diamino acids. Schreiner and Skinner (28) reported the following organic nitrogen compounds in soil: arginine, adenine, cytosine, choline, creatine, creatinine, histidine, hypoxanthine, nucleic acid, guanine, and trimethylamine.

Jodidi (16) states that nitrogen in its initial stages may be found in considerable quantities in soil. The amino acids and acid amides cannot accumulate to any extent because they are easily ammonified. The ammonia and nitrites cannot accumulate because they are readily

oxidized to nitrates which are utilized by plants or leached out of the soil if it is fallowed. He (15) analyzed a series of soil samples which showed an ammonia content of 0.0015 per cent or less and a nitric content of less than 0.01 per cent. Oven-dried soils contained 0.257 per cent or less of total nitrogen, 0.0015 per cent or less of ammonia, and 0.096 per cent or less of acid amides.

Headden (11) reported the following analyses of Colorado soils: protein nitrogen, 0.07 to 0.08 per cent; ammonia nitrogen, near 0.01 per cent; amino nitrogen, average about 0.04 per cent; nitric nitrogen, 0.05 per cent or lower; total nitrogen, mostly below 0.2 per cent with a few samples showing as much as 0.3 per cent.

Neidig and Snyder (24) found that Idaho soils contained from 0.152 to 0.886 per cent total nitrogen. Russell (27) states that the total nitrogen of arable soils is usually about 0.15 per cent, and that of pasture lands 0.03 per cent. The ammonia content of heavily dunged areas is 0.001 per cent. Rich garden soils contain 0.006 per cent of nitrates, and arable soil 0.002 per cent.

PRESENT INVESTIGATION[1]

Methods

Four strains of *Azotobacter chroococcum* were employed. Three of these were stock laboratory strains of the organism, and are designated in the tables as A1, A3, and A4. All were able to fix substantial quantities of free atmospheric nitrogen when cultivated in a medium free from combined nitrogen, A4 being the least active in this respect. The fourth strain, designated as Am, was freshly isolated from soil. It fixed free atmospheric nitrogen more actively than the other three strains. Although it exhibited the characteristic morphology of Azotobacter, it did not produce as much pigment in cultures as the three stock strains.

Experimental work in nitrogen fixation is frequently criticised on the ground that the Azotobacter cultures used have been carried in the laboratory on media containing no combined nitrogen until they have been trained to fix free atmospheric nitrogen. This objection is eliminated partially in this study by the use of strain Am, which was re-isolated several times during the progress of the work.

Ashby's (1) medium was employed for all of the experiments. Its composition is as follows:

KH_2PO_4	0.2 gm.
$MgSO_4$	0.2 gm.
NaCl	0.2 gm.
$CaSO_4$	0.1 gm.
$CaCO_3$	5.0 gm.
Mannitol	12.0 gm.
Water	1,000 cc.

The KH_2PO_4 is neutralized to phenolphthalein with 10 per cent NaOH before it is added to the rest of the medium. If a solid medium is desired,

[1] For a more detailed presentation of some phases of the investigation, the reader is referred to the doctorate dissertation of the senior author in the Yale University library.

1.5 per cent agar is added. Ashby advocated starting the cultures of Azotobacter on an agar base in the culture flasks or bottles, and after three days' incubation at the desired temperature, adding the nutrient solution in desired quantities. This method insures better seeding of the Azotobacter than can be obtained otherwise. All of the media were sterilized at 15 pounds for 20 minutes.

In the following experiments, 10-cc. quantities of Ashby's agar were placed in 200-cc. Blake culture bottles and the bottles set on edge after sterilization. An inoculum was prepared by making a suspension of a week-old culture of Azotobacter in physiological saline and adjusting the density to 5.0 in the McFarland (22) nephelometer scale. By means of a sterile pipette, 1 cc. of the suspension was transferred to the agar in a Blake bottle. After three days incubation at 28°C., 50 cc. of Ashby's nutrient solution was added.

In the experiments in which various forms of combined nitrogen were used the desired amount of the nitrogenous substance was added to both the solid and fluid forms of the Ashby medium.

Preliminary experiments determined the most favorable time and temperature for incubation to be 21 days at 28° to 30°C. The quantity of inoculum was found to be unimportant, within reasonable limits, as long as a sufficient seeding was obtained to give a satisfactory growth. The nitrogen content of the inoculum employed was not sufficient to be measurable by the Kjeldahl method and consequently did not influence the total nitrogen determinations in the cultures after incubation.

After 21 days' incubation at 28°C., determinations were made of the amount of growth, ammonia and amino acid nitrogen, and total nitrogen.

The estimates of growth were checked by a direct microscopic examination of the cultures, using the technic advised by Conn (6). Rose Bengal was employed as a stain. Direct plate examinations of the cultures for numerical determination of the bacteria present did not prove sufficiently consistent to be satisfactory, probably because of the mucous-like consistency of the Azotobacter growth.

Ammonia determinations were made with the Van Slyke and Cullen modification of the Folin air current method. Amino acid nitrogen was determined by Brown's (5) modification of the technic of Henrique and Sørensen. The estimations of ammonia and amino acid nitrogen in the cultures were begun with the hope that they could be used as measures of the nitrogen fixed by Azotobacter. These proved to be of no value in that way because no ammonia or amino acid nitrogen could be detected in cultures of the organism grown in a solution containing no combined nitrogen. In the experiments on the effect of combined nitrogen, however, both the amino acid nitrogen and ammonia tests were useful in estimating the activity of Azotobacter in attacking the nitrogen compounds.

Total nitrogen was determined by means of the Kjeldahl method. Five per cent copper sulfate was used as a catalyzer. Distillations were made into

$N/14$ sulfuric acid which was titrated back with $N/14$ sodium hydroxide, Congo Red being used as an indicator.

Experiments were conducted to determine the possible toxic properties of the nitrogen compounds towards Azotobacter. For these tests Ashby's agar containing the desired concentration of combined nitrogen was inoculated with Azotobacter and plates were poured in the usual way. The plates were incubated for one week at 28°C.

Does Azotobacter fix free atmospheric nitrogen in a definitely controlled atmosphere containing no combined nitrogen?

The first experiment was undertaken to determine the ability of Azotobacter to fix atmospheric nitrogen when cultivated in a definitely controlled gaseous environment. Bonazzi (4) was bold enough to suggest that this genus may not fix any free atmospheric nitrogen at all, but that it merely accumulates available combined nitrogen which may be present in the air as ammonia or nitrous acid. It is reasonable to suppose that a considerable quantity of these substances, considered from the standpoint of microörganic needs, may be present in the atmosphere under certain conditions. This would be especially true of a soil where organic decomposition is taking place. Although the amount of either ammonia or nitrous acid available at any one moment is probably exceedingly small, over an incubation period of 21 days, or more, Azotobacter may conceivably come in contact with sufficient nitrogen in these forms to account for the quantity found in the cultures.

The literature contains little evidence of any serious consideration having been given to the possibility that the nitrogen accumulation in cultures of Azotobacter may be due to the assimilation of combined rather than free atmospheric nitrogen. Winogradsky (35) in the isolation and study of *Clostridium pasteurianum*, aerated his cultures with air which had been passed through sulfuric acid. Beijerinck (2) comments on this practice, but apparently it has not been followed generally.

In order to investigate the ability of Azotobacter to fix free atmospheric nitrogen in the absence of any combined nitrogen in the air and in a medium free from combined nitrogen, the following procedure was adopted:

A series of cultures of the organism in Ashby's medium was incubated in sealed containers which were aerated daily with an atmosphere free from combined nitrogen.

A second series of cultures was set up in all respects like the first, but incubated in contact with ordinary room air which was supplied to the sealed containers in the same manner as the washed air in the first series.

Aeration was accomplished as follows: A 3-gallon bottle was filled with water and the water gradually replaced with air which had been drawn through a scrubbing tower filled with 10 per cent sulfuric acid to remove the ammonia, and through 25 per cent sodium hydroxide for the removal of nitrous acid that may have been present. The water was drawn from the bottle by a

siphon, the washed air being allowed to enter through a short intake tube; or by inverting the bottle and allowing the water to run out through a short tube in the rubber stopper, while the washed air entered through a long tube which extended from the stopper to above the water level.

After the 3-gallon bottle had been filled with washed air it was connected with the sealed container by means of rubber tubing, and the air from it was forced through the container by means of water siphoned into the first bottle from a second bottle of the same size.

Two plans of aeration were tried. The first was gradual and continous aeration for the period of incubation, fresh bottles of washed air being supplied once each day. This method was effective but required a complicated apparatus with a number of aerating bottles in use at the same time. The second plan was to aerate the containers more rapidly, the air being forced through once each day for a period of 20 minutes. This made it possible to aerate the containers outside of the incubator, and allowed the incubation of many more cultures at the same time than would have been possible with the first plan.

TABLE 1

Ability of Azotobacter to fix free nitrogen in an atmosphere from which all combined nitrogen has been removed

ORGANISM	NITROGEN FIXED PER 100 CC.	
	Ordinary room air	Air free from combined nitrogen
	mgm.	mgm.
A1	6.6	7.0
A3	6.4	6.4
A4	5.4	5.6
Am	8.8	8.4

The second method proved equally satisfactory and was adopted, since it allowed a more rapid accumulation of data.

It was observed that the sodium hydroxide removed the carbon dioxide from the air, and that the growth and activity of Azotobacter appeared to be greatly inhibited thereby. To counteract this influence, approximately 1 per cent by volume of carbon dioxide was added to the washed air before it was introduced into the culture containers. The influence of carbon dioxide on bacteria has been discussed by Valley and Rettger (32).

The air driven from the sealed containers during aeration was bubbled through 0.01*N* sulfuric acid in order to collect any ammonia which may have been given off from the cultures during incubation. The acid was titrated back with 0.01*N* sodium hydroxide, Congo Red being used as the indicator.

Two sets of cultures of Azotobacter were set up according to the plan outlined in the foregoing. The cultures were seeded on Ashby's agar in Blake culture bottles and incubated for three days at 28°C., after which 50 cc. of Ashby's solution was added and the cultures were incubated for 21 days at

28°C. Total nitrogen determinations were made at the end of the incubation period. Table 1 shows that the four Azotobacter strains used here are capable of fixing appreciable quantities of free atmospheric nitrogen in the absence of any combined nitrogen in the substrate and the surrounding atmosphere. Differences observed between the quantities of nitrogen fixed by the same Azotobacter strain in washed air as compared with ordinary room air are within the range of normal variation, which might be expected among cultures of the organism set up and incubated under identical conditions.

Since the foregoing experiment demonstrated the ability of Azotobacter to fix free atmospheric nitrogen in the absence of any combined nitrogen in the air or substrate, it was decided to incubate all cultures in the subsequent experiments in sealed containers which were aerated once each day with washed air.

Is Azotobacter proteolytic?

In order to test the proteolytic power of Azotobacter, inoculations of the four strains of the organism were made on Loeffler's blood serum. These were incubated for one week at 28°C. and examined. A good growth was observed in all cases, but there were no signs of liquefaction of the medium. These cultures were continued in incubation and examined from time to time until the medium became so dry that further observations were useless. The total incubation time was 40 days. At no time was there evidence of any digestion of the blood serum.

In another test the Ashby solution was employed as the basic medium and 300 gm. of gelatin to each liter added. The medium was used in tubes (slanted) and in plates. Tubes and plates were inoculated with Azotobacter and incubated at 22°C. for 20 days, after which they became too dry for satisfactory observation. Daily examination showed good growth of the organism after the third day, but there was no liquefaction of the gelatin at any time.

These tests indicate that Azotobacter is not proteolytic. The gelatin test should be especially conclusive, since inability to attack gelatin implies the lack of proteolytic power in the organism.

Influence of combined nitrogen in the substrate on growth and nitrogen fixation by Azotobacter

The chemical substances chosen for the study of the influence of combined nitrogen in the substrate on growth and nitrogen fixation by Azotobacter were as follows:

Peptone, nucleic acid, tryptophane, tyrosine, glutamic acid, aspartic acid, cysteine hydrochloride, cystine, glycocoll, creatine, creatinine, urea, guanine, ammonium sulfate, ammonium chloride, ammonium carbonate, ammonium phosphate, ammonium nitrate, sodium nitrate, and potassium nitrate.

It is obviously impossible in an investigation of this scope to determine the influence of all of the known nitrogenous compounds which may be found in soil. The compounds chosen, however, should furnish considerable evidence regarding the behavior of Azotobacter in the presence of combined nitrogen. Both simple and complex organic compounds were employed as well as some readily available inorganic substances for comparison.

The cultures were set up as indicated in the description of methods, and incubated for three days at 28°C. Then 50 cc. of Ashby's solution containing

TABLE 2

Amount of nitrogen fixed by Azotobacter in the presence of different nitrogenous compounds, and of nitrogen fixation by the same organisms in a medium having no combined nitrogen

Results are expressed as mgm. of nitrogen in 100 cc.

MEDIUM	STRAINS OF AZOTOBACTER			
	A1	A3	A4	Am
No combined nitrogen	7.0	6.4	5.6	8.4
Bacto-peptone	3.2	3.8	3.4	2.0
Nucleic acid	4.8	5.0	2.6	5.0
Tryptophane	3.1	5.2	2.4	6.0
Tyrosine	5.0	5.2	5.6	6.0
Glutamic acid	1.6	2.0	1.0	2.0
Aspartic acid	−1.2*	1.7	0.4	−0.2
Cysteine hydrochloride	1.0	0.8	0.4	0.8
Cystine	5.0	6.2	7.0	5.6
Glycocoll	3.0	2.6	3.2	2.6
Creatine	−1.8	−0.6	−0.8	−1.0
Creatinine	−2.4	−0.8	−2.6	−1.0
Urea	−2.5	2.3	−1.5	−0.7
Guanine	2.8	4.9	4.0	5.3
Ammonium sulfate	1.4	3.4	2.3	1.4
Ammonium chloride	5.1	7.8	8.2	6.3
Ammonium carbonate	6.3	7.5	5.9	3.0
Ammonium phosphate	5.0	5.5	3.8	2.6
Ammonium nitrate	6.3	8.1	6.1	1.2
Sodium nitrate	6.7	5.6	4.4	0.6
Potassium nitrate	0.0	0.6	0.0	−3.4

Indole and skatole were toxic and no growth occurred.

* The minus sign indicates that less nitrogen was recovered from the cultures after incubation than was included in the medium as combined nitrogen.

enough of the nitrogen compound employed to give the desired concentration of nitrogen was added and the cultures were incubated for 21 days at 28°C. Because of the number of compounds employed and the necessity of repeating the experiments with each several times, a standard concentration of 100 p.p.m. of nitrogen was adopted. This amount of nitrogen greatly exceeds that found in the average soil combined in any single compound.

After incubation the cultures were examined as follows:

Visual growth was observed; tests were made for ammonia and amino acids in the cultures, and for indole where the nature of the culture solution suggested their possible presence: tests were conducted for ammonia lost during incubation; finally, total nitrogen determinations were made, and the nitrogen fixed was calculated by subtracting the nitrogen content of the control cultures from that of the test cultures.

The observation of the amount of growth in the cultures may show that the increase of Azotobacter growth is greatly in excess of the increase of nitrogen

TABLE 3

Influence of combined nitrogen on the growth of Azotobacter in a fluid medium

MEDIUM	STRAINS OF AZOTOBACTER			
	A1	A3	A4	Am
No combined nitrogen	2x*	2x	2x	2x
Bacto-peptone	4x	4x	3x	4x
Nucleic acid	2x	2x	2x	2x
Tryptophane	2x	2x	2x	2x
Tyrosine	2x	2x	2x	2x
Glutamic acid	2x	2x	2x	2x
Aspartic acid	3x	3x	3x	3x
Cysteine hydrochloride	3x	3x	3x	3x
Cystine	2x	2x	2x	2x
Glycocoll	3x	3x	3x	3x
Creatine	3x	3x	3x	3x
Creatinine	3x	3x	3x	3x
Urea	4x	4x	2x	4x
Guanine	2x	2x	2x	2x
Ammonium sulfate	3x	3x	3x	4x
Ammonium chloride	4x	3x	3x	3x
Ammonium carbonate	4x	3x	3x	4x
Ammonium phosphate	4x	3x	3x	4x
Ammonium nitrate	4x	4x	3x	4x
Sodium nitrate	3x	4x	2x	4x
Potassium nitrate	3x	3x	3x	3x
Indole	00	00	00	00
Skatole	00	00	00	00

* The normal growth of each strain in the medium containing no combined nitrogen is reported in table as 2x.

fixed, or that growth has increased while nitrogen fixation is actually inhibited. The production of detectable ammonia from the nitrogenous compounds may indicate the ability of Azotobacter to attack the nitrogen compound employed in a given culture. If ammonia is liberated slowly, it may be utilized by the organism, whereas, if it is formed rapidly, it may escape from the alkaline solution during incubation. In that case, the test for ammonia lost during incubation would be useful. In cultures containing amino acids as such, in-

cluding peptone, the amino acid test should determine the amount of the amino acid remaining unused and thus indicate the ability of Azotobacter to

TABLE 4

Amount of ammonia nitrogen detected in fluid cultures of Azotobacter in the presence of combined nitrogen

MEDIUM	STRAINS OF AZOTOBACTER			
	A1	A3	A4	Am
No combined nitrogen	00	00	00	00
Bacto-peptone	1.4	3.6	2.8	1.2
Nucleic acid	Trace	Trace	Trace	Trace
Tryptophane	Trace	Trace	Trace	Trace
Tyrosine	Trace	Trace	Trace	Trace
Glutamic acid	Trace	Trace	Trace	Trace
Aspartic acid	Trace	Trace	Trace	Trace
Cysteine hydrochloride	4.2	1.4	2.1	5.6
Cystine	1.4	1.4	0.0	2.1
Glycocoll	2.1	1.4	2.1	2.8
Creatine	Trace	Trace	Trace	Trace
Creatinine	2.1	2.1	1.4	2.1
Urea	−1.7*	−0.4	−1.1	−0.4
Guanine	Trace	Trace	Trace	Trace
Ammonium sulfate	−0.8	−1.2	−0.4	−1.5
Ammonium chloride	−1.7	−1.2	−1.4	−1.4
Ammonium carbonate	0.0	−0.4	0.0	−0.4
Ammonium phosphate	−0.5	−0.5	−0.5	−0.7
Ammonium nitrate	−1.5	−0.5	−0.5	−0.8
Sodium nitrate	0.0	Trace	Trace	0.0
Potassium nitrate	0.0	0.0	0.0	0.0
Indole	0.0	0.0	0.0	0.0
Skatole	0.0	0.0	0.0	0.0

* The minus sign indicates that less ammonia was detected in the test cultures than in the control cultures.

TABLE 5

Amount of amino acid nitrogen remaining in fluid cultures of Azotobacter after 21 days' incubation

ASHBY'S MEDIUM, PLUS	AMINO ACID NITROGEN
Bacto-peptone	Trace
Nucleic acid	None
Tryptophane	One-half remained
Tyrosine	Practically all remained
Glutamic acid	One-third remained
Aspartic acid	None remained
Glycocoll	Trace

* The table represents only those cultures which had an amino acid or peptone included in the culture solution, since no amino acid nitrogen was detected in any of the other cultures of the series.

utilize amino acids. The total determination of fixed nitrogen is the main object of the investigation and needs no further explanation.

The results of the various tests are shown in the tables 2 to 6. Nitrogen fixation, which is the main objective of the experiments, is shown in table 2, growth in table 3, ammonia detected in cultures in table 4, amino acids in table 5, and ammonia lost during incubation in table 6. No indole was detected in any of the cultures.

TABLE 6

Amount of ammonia lost from fluid cultures of Azotobacter during incubation

MEDIUM	MGM. OF AMMONIA LOST PER 100 CC. OF CULTURE
No combined nitrogen	No loss demonstrated
Bacto-peptone	Average less than 0.1 mgm.
Nucleic acid	No loss demonstrated
Tryptophane	No loss demonstrated
Tyrosine	No loss demonstrated
Glutamic acid	No loss demonstrated
Aspartic acid	Average 0.8 mgm.
Cysteine hydrochloride	No loss demonstrated
Cystine	No loss demonstrated
Glycocoll	No loss demonstrated
Creatine	Average 3.2 mgm.
Creatinine	Average 0.4 mgm.
Urea	Average 1.7 mgm.
Guanine	No loss demonstrated
Ammonium sulfate	Average less than 0.1 mgm.
Ammonium chloride	No loss demonstrated
Ammonium carbonate	No loss demonstrated
Ammonium phosphate	Average less than 0.1 mgm.
Ammonium nitrate	Average 0.4 mgm.
Sodium nitrate	No loss demonstrated
Potassium nitrate	No loss demonstrated
Indole	No loss demonstrated
Skatole	No loss demonstrated

REVIEW OF DATA

Of the organic nitrogenous compounds employed, nucleic acid, tryptophane, and tyrosine did not exert any marked effect on the amount of nitrogen fixed by Azotobacter; nor was there any noticeable influence on the quantity of growth. The slight depression of nitrogen fixation suggests that the compounds may have been slightly utilized to furnish nitrogen for the use of the organism.

Peptone had a marked effect, resulting in an increase of growth and a definite depression of nitrogen fixation, as was shown by comparison with the nitrogen fixation by the same organism in a medium that was free from combined nitrogen. Commercial peptone contains many simple decomposition products of

protein digestion which may have been responsible for the pronounced influence on Azotobacter growth and activity.

Guanine, cystine, and glutamic acid depressed nitrogen fixation. This was especially true of glutamic acid. Growth was not influenced. There was no other evidence that any of these compounds was attacked by the organism. Cystine exercised the least effect of the three. It was probably present in very small quantities because of its limited solubility in the medium.

Aspartic acid, cysteine hydrochloride, glycocoll, creatine, creatinine, and urea all produced increased growth and caused marked depression of nitrogen fixation. With the last three there was actual loss of some of the original nitrogen of the medium.

Indole and skatole, in the concentrations employed, were decidedly toxic to Azotobacter.

Apparently all of the inorganic compounds were available for use by Azotobacter, growth in the liquid cultures being greatly stimulated by their presence. Nitrogen fixation was almost equal to that observed in media containing no combined nitrogen, with the exception of the experiments in which ammonium sulfate and potassium nitrate were employed. A marked exception to this rule was the behavior of organism Am. Nitrogen fixation by this strain was decidedly reduced by all of the inorganic nitrogenous salts except ammonium chloride. With potassium nitrate some of the original nitrogen was lost in cultures of Am. This loss was the greatest noted in any of the cultures in which combined nitrogen was employed.

A comparison of the growth of Azotobacter in the presence of inorganic nitrogenous salts with the amount of nitrogen fixed indicates that the metabolic efficiency of the organism was greatly inhibited.

Definite quantities of ammonia were detected in cultures containing peptone, cysteine hydrochloride, glycocoll, and creatinine. It seems probable that this ammonia resulted from decomposition of the compounds by Azotobacter, and that if the evolution of ammonia is slow enough, the organism will utilize it for conversion to protoplasmic nitrogen. No ammonia was observed in cultures containing creatine, in spite of other evidence that Azotobacter had utilized this substance.

The loss of ammonia from cultures containing creatine was the greatest observed among the experiments. Probably the material was attacked so vigorously that the ammonia escaped from the medium before it could be utilized. Some ammonia was also lost during incubation from cultures containing aspartic acid, creatinine, urea, and ammonium nitrate.

GENERAL DISCUSSION

The ability of Azotobacter to fix atmospheric nitrogen in the complete absence of combined nitrogen was definitely demonstrated with four different strains in the first experiment. It seems reasonable to assume that other

strains of the organism would possess the same power. Although it is improbable that laboratory air, under ordinary conditions, would contain a measurable quantity of ammonia or nitrous acid at any one moment, the exposure of the cultures to minute amounts of these substances over a 3-week incubation period may have a decided influence on the behavior of the organisms. The present experiments were carried out, therefore, in an atmosphere free from combined nitrogen.

In considering the influence of combined nitrogen in the medium on growth and nitrogen fixation by Azotobacter, the results obtained do not agree entirely with those reported by Hills (13), who found that nitrogen fixation was stimulated by the presence of ammonium, sodium, and potassium nitrates. No such stimulus was observed in this investigation. Hills' experiments were carried out in soil which naturally may be expected to contain some nitrates, with a consequent complication of results. Another factor in his work which seems worthy of consideration is the interpretation of the relation of the quantity of growth to nitrogen fixation. He reported that growth was stimulated by the presence of nitrates. If the total-nitrogen figure is considered alone, it can be said that nitrogen fixation was stimulated. If the efficiency of the individual organism is considered, then it is logical to conclude that the nitrogen fixation by each individual cell was inhibited.

Reed and Williams (25) found that urea, glycocoll, and creatinine were only slightly depressive in their effect on nitrogen fixation. In the present investigation creatinine and creatine were both readily utilized and the nitrogen-fixing function entirely suspended. Reed and Williams do not appear to offer any evidence, other than the effect on nitrogen fixation, to indicate that Azotobacter is able to attack these substances.

Zoond (37) stated that minute traces of potassium nitrate, tyrosine, and Bacto-peptone depressed nitrogen fixation by Azotobacter cultures. The present findings do not agree in the instance of tyrosine, which had little or no effect.

The possible influence of some other factor than nitrogen, such as the sulfur of ammonium sulfate, cysteine, and cystine, must be considered in the interpretation of results. Greaves (8) states that azofiers require sulfur for the formation of the protein material of their bodies.

Bonazzi (4) states that it is a common experience to observe an increase of Aztobacter growth in the presence of nitrates. On the other hand, Waksman (33) suggests that among the reasons for the decreased fixation of nitrogen by Azotobacter in the presence of nitrates is the toxic effect which the nitrate salts exert on Azotobacter growth. The results obtained in this investigation indicate that nitrates are not toxic in quantities likely to be encountered in normal soil. Hills' (13) work agrees with this statement.

It was observed in these experiments that indole and skatole are toxic to Azotobacter in the concentrations used. Reed and Williams (25) report the same result with skatole. They do not report any experiments with indole.

The fact that these compounds are toxic for Azotobacter in relatively small quantities suggests the possibility that they may exercise the same effect in soil at times, as they are likely to be present as decomposition products of protein.

A survey of the findings of the foregoing experiments suggests that Azotobacter will utilize available combined nitrogen where possible instead of following the more laborious process of fixing free nitrogen. One is reminded of the opinion expressed by Bonazzi (4), that Azotobacter has the power to fix free atmospheric nitrogen, but that the function is only operative in case of necessity, and not always, or even often, for the benefit of man.

Table 2 shows that the behavior of strain Am in the presence of ammonium carbonate, ammonium phosphate, ammonium nitrate, sodium nitrate, and potassium nitrate is very different from that of the other Azotobacter strains employed. This fact suggests that care should be used in making general assertions regarding the behavior of Azotobacter from data obtained with a few strains.

SUMMARY

Azotobacter appears to be able to fix substantial quantities of free atmospheric nitrogen when cultivated in a medium which is free from combined nitrogen and in an atmosphere which is free from ammonia and nitrous acid.

Azotobacter did not attack blood serum or gelatin.

Nucleic acid, tryptophane, and tyrosine depressed nitrogen fixation slightly, and did not influence the growth of Azotobacter.

Peptone increased the growth of the organism, but decreased nitrogen fixation.

Glutamic acid, aspartic acid, cysteine hydrochloride, and glycocoll appeared to be readily utilized by Azotobacter. Nitrogen fixation was definitely depressed, however, and in some cases some of the original nitrogen was lost. Growth was increased in all cultures.

Cystine had little effect, probably because of its limited solubility in the medium.

Creatine, creatinine, and urea caused increased growth in most of the cultures, but depressed nitrogen fixation almost entirely, In some of the cultures there was loss of some of the original nitrogen. Guanine caused depression of nitrogen fixation, but did not influence growth.

Indole and skatole were toxic in concentrations as low as 50 p.p.m. of nitrogen equivalent.

Ammonium sulfate and potassium nitrate depressed nitrogen fixation and at the same time increased growth.

Ammonium chloride, ammonium carbonate, ammonium phosphate, amonium nitrate, and sodium nitrate produced a greatly increased growth in a majority of the cultures, and only a very slight depression of nitrogen fixation,

except in some of the cultures of strain Am. When the amount of growth is correlated with nitrogen fixation, a relative depression of fixation is apparent.

The following general observations seem to be justified:

The more complex organic compounds, except peptone, do not seem to be actively attacked by Azotobacter, and consequently the growth and nitrogen-fixing activity of the organism are not much influenced. The presence of simple nitrogenous compounds in commercial peptone is in all probability responsible for the results observed.

The simpler organic substances, including the lower amino acids, and the inorganic compounds seem to be utilized easily by Azotobacter, with the result that growth is increased and nitrogen fixation either relatively or actually depressed.

Where there is adequate evidence that the combined nitrogen is available for Azotobacter, it appears to be used in preference to the more laborious process of fixing free atmospheric nitrogen.

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A LARGE SAND CULTURE APPARATUS

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A large sand culture apparatus designed to permit the growing of comprehensive groups of crop plants to mature stages under comparable conditions is here described. Empirical proofs showing that plants differ greatly in their nutritional requirements, tolerances, and absorption rates, indicate a need for a suitable method for obtaining comparative information of this kind for the more important plants and the essential and toxic elements. The apparatus here described has been used for the past year at this laboratory in a study of the growth reactions of crop plants to different concentrations of boron. The equipment has been found satisfactory for the purpose indicated and it seems probable that it may be applicable to other nutritional studies as well.

In the boron work, outstanding differences have been found not only in the requirements and tolerances of the plants compared but also in the amounts of boron which the different plants absorbed. Information so gained has proved to be of direct value in itself and it is providing a basis for the interpretation of field observations. A knowledge of the relative behaviours of the different plants is likewise helpful in the selection of test plants best suited for critical studies of significant physiological differences and of factors which influence boron tolerance.

Many of the sand culture methods which have been described in the past require an apparatus which is not only elaborate in construction but which is exacting in the amount of attention and time necessary for its maintenance and manipulation. None of the methods with which the writer was familiar avoided the difficulties encountered in the use of a large number of individual pots. If individual pots are used for each of the different plants compared it is practically certain that the concentration of the ion of interest will vary around a different mean in each of them. These inequalities can be reduced by the use of large volumes of culture solution but the replacements of the culture solutions necessarily must be frequent since plants of different size and kind have different transpiration and absorption rates. The sand culture beds shown in figure 1 and plate 1 provide a rapid and simple method for the daily or more frequent replacement of culture solutions, and in the large sand beds space is available for extensive spreading and overlapping of roots. This overlapping of roots in the culture medium, which is common to all of the plants

receiving the same treatment, serves to equalize the concentration of the solution available to the different plants between the successive replacements.

Information of the general type indicated may be obtained in some cases from differently treated field plots, but under field conditions there are numerous uncontrolled factors, and uncertainties are introduced by soil heterogeneity, chemical reactions in the soil, and by the vertical distribution of the element in question in relation to the root systems of the different plants. Soils with initial characteristics which cause them to be at once suitable for studies on the effects of both a minimum and a large amount of a particular element are usually difficult to find.

SAND CULTURE APPARATUS

A set of five sand beds is shown in plate 1 as they have been set up and used at this laboratory. A line sketch illustrating one of the beds with its solution barrels and plumbing is shown as figure 1.

In operation the procedures are as follows: A cloth bag is suspended through the opening of the lower of the two barrels and into this bag are placed the weighed portions of the different salts required for the nutrient solution. Water is then allowed to flow from a hose through this bag until the salts are dissolved and the barrel is filled. It is desirable to add the readily soluble calcium nitrate to the bags after the other salts have been dissolved. With the valves at *A* and *B* closed the contents of the lower barrels are drawn into the upper barrels by exhausting the air from the latter by means of an air line connected to a filter pump on a faucet at one of the laboratory sinks. This air line is common to each of the five barrels and no valves are used. About one hour is required for lifting the solutions from the lower to the upper barrels. To flood the sand beds the valves at *B* and *C* are closed and those at *A* are opened. The whole of the solution in the upper barrel can be passed through the sand by opening the valves at *C* slightly as soon as the culture solution has reached the surface of the sand. The by-pass, with valve *B*, is necessary only when the beds are flooded from below, which should be done occasionally to prevent the sand from becoming compacted in the beds.

The frequency with which the culture solutions are discarded, by allowing them to escape through valve *D*, is dependent upon the character of the experiments. As the boron experiments are conducted the beds are flooded daily with the same solution for from 12 to 8 days during the seedling stages but as the plants become larger and their transpiration rates increase, new solutions are substituted for the old as often as every five days. It has been found that the original boron concentrations can be maintained in the solutions for a week or longer by adding sufficient water to replace three-quarters of the daily transpiration. The rate of boron absorption by plants is dependent, however, upon a number of factors and though good growth can be maintained for considerable periods by replenishing the solution with water it has not been considered

desirable in the boron experiments to add more than a total of 50 gallons of water to any of the solutions before they are discarded.

Daily conductivity measurements made on samples collected from the lower barrels provide a convenient method for following the changes in concentrations which take place in the culture solutions with use. Or, if an easily determinable ion is of primary interest, a daily analysis will indicate the amount of water, or of the element, which should be added to a solution to bring it

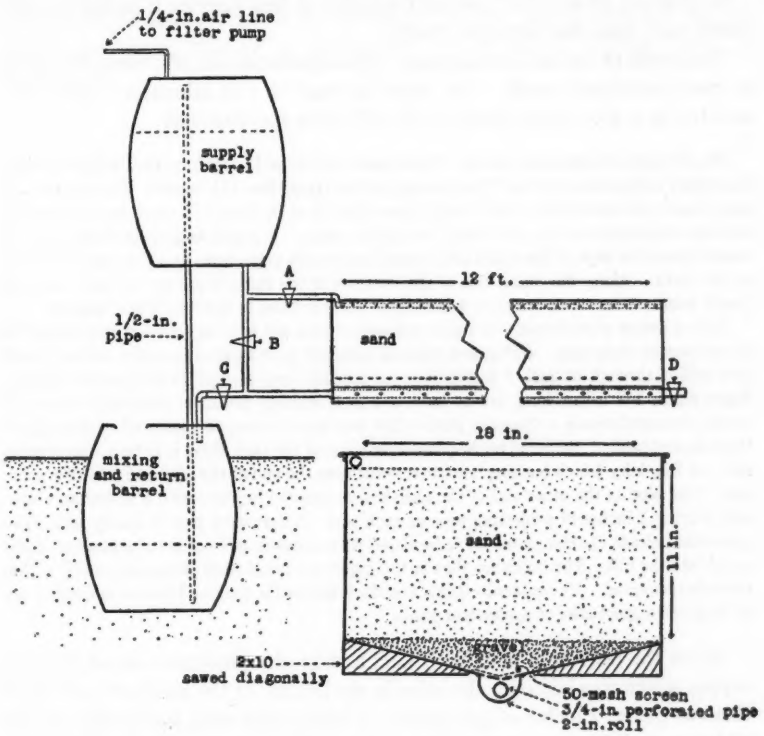

FIG. 1. SECTION OF SAND CULTURE APPARATUS, SHOWING SANDBED, SOLUTION BARRELS, AND PLUMBING

back to its original strength. Although it has not been tested, it appears probable that a sample collected from valve *D* before *C* is opened should be closely representative of the solution displaced from the sand after a day's use.

With beds of the dimensions indicated, approximately 25 gallons of nutrient solution is required to flood the sand in a bed after it has been once brought to saturation and drained. Starting with an air-dried sand, a full barrel, 50 gallons, is needed for the first flooding.

A culture solution which has been used and found satisfactory for the growth and maturity of most of the crops tested is as follows:

$Ca(NO_3)\cdot_2 4H_2O$	6 millimoles per liter,	268.1 gm. per 50 gallons
$MgSO_4\cdot 7H_2O$	3 millimoles per liter,	139.9 gm. per 50 gallons
KH_2PO_4	3 millimoles per liter,	77.3 gm. per 50 gallons
Mn as $MnCl_2$	0.5 p.p.m.,	0.22 gm. per 50 gallons
B as H_3BO_3	1 p.p.m.,	1.08 gm. per 50 gallons

In addition, 20 cc. of a 5 per cent solution of iron tartrate is added to each barrel each time the beds are flooded.

The details of size and arrangement of the apparatus can, of course, be varied to meet individual needs. The tanks as used in this laboratory were constructed in a sheet metal shop on the following specifications:

Five 20-guage, rust resistant tanks.—These tanks are to be 12 feet long and 18 inches wide. The depth at the sides is to be 11 inches and at the center line 12½ inches. The top of each tank is to be reinforced with 1-inch angle iron which is to be riveted in place and covered by bent-over extensions of the galvanized iron of the tanks. A 1-inch angle iron tie rod is to be placed across the tops of the tanks and riveted to the side angle irons at 4 feet from each end of the tanks. Along the center line of the bottoms of the tanks there are to be 3 rows of ¼-inch holes. These rows are to be ½-inch apart and the holes in the rows 2 inches apart.

Roll on bottom of each tank.—A 2-inch galvanized iron roll is to be soldered and riveted to the bottom of each tank. *Galvanized pipes in rolls.*—A ¾-inch galvanized pipe with a ¼-inch hole drilled through at each 8 inches is to be supplied and secured in each roll as follows: These pipes, 148 inches long, are to have a 2 inch running thread at each end which will extend through holes in a 16-guage plate which has been riveted and soldered to the ends of the rolls and tanks. Soldered to the plate at one end of the tank there is to be a 1 inch waste nut. A 1 inch by ¾-inch bushing is to be screwed over the end of the pipe and into this waste nut. The pipe at the other end of the tank will be secured in place with a gasket and locknut, leaving 2 inches of projecting pipe at each end. Before each pipe is finally secured in place, the holes in the end plates are to be closed with stoppers and a quart of asphaltum paint pored into the roll. The tanks are then to be tipped and rolled until all exposed metal within the rolls is covered. The galvanized pipe and the inside of the tank and the outside seams are to be given a good coat of asphaltum paint.

Before the beds were filled with sand a strip of asphaltum painted 50-mesh copper screen was laid over the holes in the bottom of the tanks and on top of this was placed 2 inches of pea gravel. Clean quartz sand, sufficiently fine for all but a small fraction to pass a 40-mesh screen, was used to fill the beds. The screen and gravel used in the bottoms of the tanks effectively prevent sand from passing into the rolls or drain pipes. The ½ inch galvanized pipe at the surface of the sand is provided with an ⅛ inch hole at each 6 inches. These holes are set at an angle of 45° with the sand.

The cost of each of the units including the tank, two 50-gallon barrels, plumbing, plumbing supplies, supports, and labor of setting up, was $75.00, of which amount the cost of each of the tanks constructed according to the above specifications was $35.00. The beds were set on frames constructed of 2 inch by 6 inch lumber, supported by hollow tile as shown in plate 1. The barrels

were mounted on a frame constructed of 2 inch by 12 inch lumber similarly supported with hollow tile.

The apparatus described was designed for experimental use in the open but it appears that it might be well suited for purposes of propogation in greenhouses in place of soil benches, particularly when a ready means of soil sterilization by steam or chemicals is essential. For such a purpose the supply barrels could be located in a corner of the headhouse and if the water was regularly added to replace that lost by transpiration, only an occasional renewal of the entire culture solution would be necessary. Under such conditions the size of the sand beds, relative to the capacity of the supply barrels, could be increased.

PLATE 1

SAND BEDS AS SET UP AND USED AT THE RUBIDOUX LABORATORY, RIVERSIDE, CALIF.

The culture solution used in the bed in the foreground contained 25 p.p.m. of boron. This quantity of boron was sufficient to kill a number of the plants tested and to reduce greatly the growth of many.

LARGE SAND CULTURE APPARATUS

FRANK M. EATON

PLATE 1

BOOK REVIEWS

Tropical Soil Forming Processes and the Development of Tropical Soils—With Special Reference to Java and Sumatra. By E. C. J. MOHR.

This was originally published in Dutch, but has been translated to the English by Dr. Robert L. Pendleton, professor of soil technology, College of Agriculture, University of the Philippines, and published in mimeographed form as Experiment Station Contribution No. 655. It consists of 213 pages including an excellent index. The first 52 pages discuss processes of soil formation under tropical conditions. The rest of the publication describes the character of the soils together with a discussion of their formation throughout Java and Sumatra.

CHARLES F. SHAW.

Principles of Agrobiology, or the Laws of Plant Growth in Relation to Crop Production. By OSWIN W. WILLCOX, Ph.D. Palmer Publishing Corp., Inc., 153 Waverly Place, New York, 1930. Cloth, 96 pp., double column, figs. 54.

In this book, designed as a text for practicing agronomists and teachers and students of crop nutrition, the author erects agrobiology as an essentially new science covering "that division of agricultural science which considers the general external relations of crop plants to their environments and their reactions as massed colonies to the action of growth factors." The laws referred to in the subtitle are the laws of: constancy of types; definite growth powers; universality of essential growth factors; constancy of the action of growth factors; joint action of growth factors; definite optima; diminishing increments of yield; increasing increments of yield; concentration law of growth factors; logistic law of crop growth; action law of growth factors; effect law of growth factors; homologous yield curves; conflicting attributes of growth factors; the real law of the minimum; calorific duty of vegetable nitrogen; and crop-fertilizer ratios. All of these laws are objectively illustrated by examples from crop records and large-scale investigations. Concepts new to the literature of crop growth are the limits to the action of growth factors and the corresponding limits to crop growth; the differentiation of subultra and perultra crop plants; the definition of perfertile soil, perultimate yield, and satiation pressure (growth inertia); the absolute scale of the growth power of plants; the calorific duty of vegetable nitrogen; and a plant physiological explanation for the law of diminishing increments of yield. Quite enough, seemingly, to

justify the separation of agrobiology as a distinct and entirely new branch of the science of plant life. Fertilizer statics, or the maintenance of soil fertility, is treated in the light of the Mitscherlich effect law. The mathematics of the Mitscherlich and the Spillman yield equations and their practical applications are treated in an appendix.

M. LUDTKE.